ISOLATION AND CHARACTERIZATION OF GROUP A STREPTOCOCCAL Fc RECEPTORS

By

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Major Department: Immunology and Medical Microbiology

An immunoblotting technique was developed to select a group A streptococcal strain rich in Fc receptors. A high Fc receptor-positive strain was selected and used as the source for the isolation of two functionally active Fc receptors. A variety of extraction techniques were compared including 1) heat extraction at neutral, acid or alkaline pH, 2) treatment with the enzymes mutanolysin, hyaluronidase, trypsin, papain or phage lysin, or 3) autoclaving or heating in the presence of sodium dodecyl sulfate. The most homogeneous receptor was recovered following heat extraction and contained two molecular weight forms. One form had a molecular weight of 56,000 daltons and the other form had a molecular weight of 38,000 daltons. The 56,000 dalton Fc receptor was capable of reacting with human IgG subclasses 1, 2, and 4, pig IgG and rabbit IgG. The 38,000 dalton Fc receptor could only bind human IgG subclass 3. The two Fc receptors could be separated by binding to and elution from a column of immobilized human IgG_3 which resulted in the isolation of the 38,000 dalton Fc receptor. The

unbound material from the immobilized IgG_3 column was applied to an immobilized column of human IgG and the 56,000 dalton Fc receptor was recovered following elution. Both Fc receptors were antigenically related. Monospecific antibodies prepared against either the 56,000 dalton Fc receptor or the 38,000 dalton Fc receptor demonstrated reactivity with both molecular weight forms. Both group A streptococcal Fc receptors were found to be antigenically and physicochemically distinct from either the type I receptor found on the majority of Staphylococcus aureus strains or the type III Fc receptors found on the majority of group C streptococcal strains.

The distribution of Fc receptors on group A streptococci recovered from patients who developed post-streptococcal glomerulonephritis was tested. A high incidence of Fc receptor positive nephritogenic strains were found, but an absolute correlation between Fc receptor expression and pathogenic potential could not be established.

CHAPTER ONE
INTRODUCTION

Certain bacteria are capable of interacting with immunoglobulins in two distinct ways. One involves a specific antigen-antibody reaction in which the F(ab) portion of the immunoglobulin molecule participates. This interaction mediates the clearance of bacteria from the host. The second interaction involves the Fc portion of the immunoglobulin molecule. It has been reported that certain staphylococci (Jensen, 1958; Forsgren and Sjoquist, 1966) and streptococci (Kronvall, 1973a) have surface receptors that are capable of reacting with the Fc region of certain classes and subclasses of immunoglobulins. Functional studies of their reactivity with different species and subclasses of IgG have suggested that five distinct bacterial Fc receptor activities exist (Myhre and Kronvall, 1981). These are designated as types I through V.

Staphylococcal Protein A - The Type I Fc Receptor

Physicochemical Properties

The most extensively studied Fc receptor is the type I receptor isolated from Staphylococcus aureus and designated protein A. Protein A has been reported to be produced and or secreted by approximately 90% of all staphylococci studied (Langone, 1982a; Sperber, 1976); however, marked quantitative differences exist between different strains. The most widely studied Staphylococcus aureus Cowan I strain is rich in surface protein A whereas the Staphylococcus aureus Wood 46 strain

expresses very low levels (Freimer et al., 1979; Reis et al., 1984a). Isolation of protein A by lysostaphin extraction results in a homogeneous product which is composed of a single polypeptide chain with a molecular weight of 42,000 daltons (Sjoquist et al., 1972, Bjork et al., 1972). The Fc binding part of the protein consists of four repetitive subunits. Recently, the gene for protein A has been identified and cloned (Uhlen et al., 1984; Duggleby and Jones, 1983; Lofdahl et al., 1983). The DNA sequence has revealed a fifth region, homologous to the four repetitive subunits. This region, however, does not appear to bind the Fc region of immunoglobulins. Protein A has an elongated structure which results in anomalous estimates of molecular weight by gel filtration (Bjork et al., 1972). It has been purified in high yields from bacterial culture supernatants, and extracts of staphylococci, by affinity chromatography, using columns of immobilized rabbit or human IgG (Kronvall, 1973b).

Biological Properties

The binding of protein A to the Fc region of IgG mediates a variety of biological activities. Complexes between protein A and IgG are capable of activating the classical complement pathway (Stahlenheim et al., 1973). Addition of protein A to human (Kronvall and Gewurz, 1970; Stahlenheim et al., 1973), guinea pig (Sjoquist and Stahlenheim, 1969), rabbit (Stahlenheim et al., 1973), dog or pig serum (Kronvall and Gewurz, 1970) was shown to deplete complement activity. This depletion depended on the amount of protein A relative to IgG. Langone et al. (1978a,1978b) demonstrated that protein A and IgG formed complexes that behaved functionally like IgM in their interaction with both whole complement and purified C1. The molecular formula of these

IgM-like complexes is $[(IgG)_2PA]_2$ (Langone et al., 1978b; Hanson and Schumaker, 1984). Several studies have shown that formation of these complexes between protein A and IgG inhibited phagocytosis of Staphylococcus aureus, by suppressing the opsonizing ability of complement (Dossett et al., 1969; Forsgren and Quie, 1974; Peterson et al., 1977; Verhoef et al., 1977; Musher et al., 1981).

Protein A is also capable of stimulating T- and B-cell mitogenesis. The nature of this response, however, depends on whether protein A is added in a soluble or insoluble form. Several studies have shown that soluble protein A can act as a potent T-cell mitogen, but can only activate B-cells in the presence of helper T-cells (Gugliemi and Preud'Homme, 1980; Schuurman et al., 1980; Dosch et al., 1980). Smith et al. (1983) suggested that the T-cell mitogenic activity was not associated with protein A, but was due to a bacterial exotoxin.

In contrast to soluble protein A, protein A on intact bacteria, or bound covalently to sepharose or sephadex particles, is primarily a T-cell-independent, B-cell mitogen (Romagnani et al., 1980; Ruuskanen et al., 1980; Pryjma et al, 1980a,b). Insoluble protein A can stimulate the secretion of IgM, IgG, and IgA by both peripheral blood lymphocytes (Muraguchi et al., 1980; Romagnani et al., 1980; Gausset et al., 1980), and spleen cells (Ringden et al., 1977).

In vivo studies have shown that protein A mediates immune reactions in a manner similar to that of antigen-antibody complexes. Some of these effects appear to involve complement activated by the protein A-IgG complexes (Lawman et al., 1984). Anaphylaxis-like reactions and Arthus reactions are seen when as little as 10 µg of protein A is injected intradermally in guinea pigs (Gustafson et al.,

1968). Larger amounts of protein A (500-1000 μg) cause fatal anaphylactic shock. In humans, an intradermal injection of 10 μg of protein A causes wheal and erythema reactions (Martin et al., 1967).

Streptococcal Fc Receptors - Type II Through Type V

Certain strains of group A, C, and G streptococci have been reported to have Fc receptors analogous to protein A (Kronvall, 1973a). This was first recognized by the ability of these organisms to agglutinate red blood cells sensitized with a sub-agglutinating dose of antibody (EA), indicating the presence of an IgG Fc receptor on the surface of these bacteria (Kronvall, 1973a). This Fc reactivity was further confirmed by measuring non antigen-specific binding of radiolabeled human myeloma IgG to these bacteria. Based on the ability of sera from different animal species to inhibit the binding of radiolabeled human IgG to a variety of bacteria, several distinct types of streptococcal Fc receptors (FcR) have been identified (Myhre and Kronvall, 1977). The receptor characteristic of group A streptococci is the FcR type II. Groups C and G streptococci carry a common, or related receptor, designated type III. Class IV FcR is found only on bovine group G β-hemolytic streptococci, and type V is found on certain strains of Streptococcus zooepidemicus (Myhre and Kronvall, 1981). See Table 1-1.

Only one of the four types of streptococcal Fc receptors has been purified and characterized. This is the type III receptor found on certain group C and group G streptococci. Heat treatment (Freimer et al., 1979), hot acid extraction (Havlicek, 1978; Muller and Blobel, 1983), phage lysis (Reis et al., 1984a; Christensen and Holm, 1976), papain (Bjorck and Kronvall, 1984), trypsin or mutanolysin digestion

TABLE 1-1

Species and Subclass IgG Reactivities of Bacterial Fc Receptors[a]

Immunoglobulin		IgG Fc Receptor Type[b]				
		I	II	III	IV	V
Human	IgG_1	+++	+++	+++	+	+++
	IgG_2	+++	+++	+++	-	+++
	IgG_3	-	+++	+++	-	-
	IgG_4	+++	+++	+++	+	+++
Mouse	IgG_1	+	-	+	NT[c]	NT
	IgG_{2a}	+++	-	+++	NT	NT
	IgG_{2b}	+++	-	+++	NT	NT
	IgG_3	+++	-	+++	NT	NT
Cow	IgG_1	-	-	+++	-	-
	IgG_2	+++	-	+++	-	+
Sheep	IgG_1	-	-	+++	-	-
	IgG_2	+++	-	+++	-	+
Goat	IgG_1	+	-	+++	-	+
	IgG_2	+++	-	+++	-	+
Horse	IgG(ab)	+	-	+++	-	+
	IgG(c)	+	-	+++	+	+
	IgG(T)	-	(+)	(+)	(+)	(+)
Rabbit	IgG	+++	+++	+++	NT	+++
Guinea Pig	IgG	+++	-	+++	NT	NT
Rat	IgG	-	-	-	NT	-
Dog	IgG	+++	-	-	NT	-
Cat	IgG	+++	-	-	NT	-
Pig	IgG	+++	+++	+++	NT	+++
Chicken	IgG	-	-	-	NT	NT

+++ = Indicates strong reactivity
+ = Indicates low reactivity
(+) = Weak, atypical reactivity
a = Summarized from Kronvall, 1973a; Myhre and Kronvall, 1977, 1980a,1981; Reis, 1984c.
b = See text
c = NT, Not tested

(Reis et al., 1985) have been used to isolate this Fc receptor. Digestions with papain or trypsin yield a homogeneous product with molecular weights of 29 K with papain, or 40 K with trypsin. Considerable heterogeneity in size of the type III Fc receptor, ranging from 30,000 to 100,000 daltons, is observed with the other extraction procedures; however, all molecular weight species are antigenically related (Reis et al., 1984a), indicating that they are oligmers or that cell wall components are attached. The streptococcal FcR type III has a very broad reactivity, including all four subclasses of human IgG and many other mammalian classes and subclasses (Myhre and Kronvall, 1980b; Myhre and Kronvall, 1981; Reis et al., 1984b; Boyle, 1984). The biological properties of the type III receptors have not been extensively characterized.

Little information is available on the type II FcR found on certain group A streptococci. Attempts to isolate this receptor have met with limited success for several reasons. Less than 50% of the group A streptococcal strains tested have detectable Fc receptors (Kronvall, 1973a; Freimer et al., 1979), whereas, the type III Fc receptor is found on greater than 80% of the group C and group G streptococcal strains tested (Myrhe and Kronvall, 1979; Myrhe and Kronvall, 1980b). The type I Fc receptor is found on approximately 90% of all Staphylococcus aureus strains tested (Langone, 1982a; Sperber, 1976). The amount of Fc receptor on the surface of positive strains of group A streptococci is much less than that found of the surface of Staphylococcus aureus or group C streptococci (Christensen and Oxelius, 1974; Freimer et al., 1979; Reis et al., 1983). In addition to low amounts of Fc receptors on group A streptococci, these Fc receptors

have been reported to be unstable during subculture (Freimer et al., 1979). Havlicek (1978) has reported that Fc receptors are found more frequently on fresh isolates than on freeze-dried strains. Only 30% of the freeze-dried strains had Fc receptors, while all of the fresh isolates tested in this study had detectable Fc receptor activity.

The type II Fc receptor, unlike protein A or the type III Fc receptor, is restricted in its reactivity with mammalian immunoglobulins, reacting only with the four subclasses of human IgG, rabbit IgG, and pig IgG (Myhre and Kronvall, 1977;1980a,b) (see Table 1-1). It has recently been reported that, based on morphological evidence, different types of IgG-Fc receptors exist on group A streptococci (Wagner et al., 1983). Wagner's electron microscopy studies show that ferritin-conjugated IgG from various species result in different labeling patterns. Studies on the inhibition of binding of homologous versus heterologous IgGs are also in agreement with the existence of more than one type of IgG Fc receptor on the same strain.

The group A streptococcal IgG Fc receptors are distinct from several cell wall constituents, including the M-protein, group carbohydrate, peptidoglycan (Christensen et al., 1979), and lipoteichoic acid (Schalen et al., 1980). In addition to IgG Fc receptors, some strains of group A streptococci are capable of binding the Fc region of human IgA through a distinct receptor (Christensen and Oxelius, 1975; Kronvall et al., 1979; Schalen et al., 1980).

Fc Receptors as Virulence Factors

The involvement of Fc receptors in the pathogenicity of disease is unknown, although some evidence suggests that there is a correlation between the virulence of certain group A streptococci and their ability to bind the Fc portion of human IgG (Burova et al., 1980,1981). Serial

mouse passages of various group A strains have been reported to result in the selection of highly virulent variants. A majority of these strains have enhanced expression of certain surface proteins, including IgG Fc receptors, indicating the possibility of an association between Fc receptor activity and virulence (Burova et al., 1980,1981). Recent evidence indicates that group A streptococcal FcR activity can be induced, or its expression enhanced, in association with other factors that are known to relate to virulence. The role of plasmids in the expression of anti-phagocytic activity, opacity factor, and IgG and IgA Fc receptors has been explored (Burova et al., 1983). Burova has found that these factors may be triggered by insertion of plasmid DNA into the bacterial chromosome. Plasmid control of the group A Fc receptors offers an explanation of why these receptors are lost during subculturing. Before the importance of Fc receptors as virulence factors can be critically assessed, however, this receptor will have to be isolated, and methods developed to quantify them on fresh isolates and within immune complexes.

Practical Applications of Bacterial Fc Receptors

The ability of certain bacterial surface proteins to react selectively with the Fc portion of immunoglobulin molecules has been utilized in a variety of immunological procedures primarily for purifying and quantifying reactive classes and subclasses of IgG (Langone, 1982b; Boyle, 1984; Goding, 1978). These receptors can be labeled to high specific activity or immobilized without loss of Fc binding activity and can be used to detect and quantify antigens, antibodies, or antigen-antibody complexes (Langone et al., 1979; Gee and Langone, 1981). Currently, by using type I and type III bacterial

Fc receptors, virtually any antibody of the IgG class, with the exception of those raised in birds or rats, can be detected. This enables a single tracer to be used in many assays for different antigens. In addition, binding of an electron dense ligand to Fc receptors can be used in a variety of techniques for locating and quantifying antigen-antibody complexes (Goding, 1978; Gee and Langone, 1981). Isolation of the type II, type IV, and type V bacterial Fc receptors with restricted reactivities would enable techniques to be developed that can focus on a narrower range of immunoglobulin species, classes and subclasses.

Summary

The binding of immunoglobulins via the Fc region to certain bacteria has been known for several years. Several studies involving the binding of various species' classes and subclasses of immunoglobulins to staphylococci and streptococci have determined that at least five distinct bacterial Fc receptors exist. Only two of these bacteria Fc receptors, however, have been purified to homogeneity and characterized. These receptors are the type I Fc receptor from Staphylococcus aureus and the type III Fc receptor, which is found on certain strains of group C and group G streptococci. The scant amount of information on the other bacterial Fc receptors is due in large part to the failure to identify bacterial strains with high levels of stable Fc receptors type II, IV or V on their surface.

The involvement of these Fc receptors in the pathogenicity of disease is unknown. Protein A has been shown to activate complement and cause B-cell mitogenesis *in vitro* and immediate-type hypersensitivity reactions *in vivo*. Little is known, however, about the biological properties of the streptococcal Fc receptors and whether they are

involved in the pathogenicity of disease. Some evidence suggests that there is a correlation between the virulence of certain group A streptococci and their ability to bind the Fc region of human IgG. Until the group A streptococcal Fc receptors are purified and the biological activities explored, however, the importance of these receptors in the pathogenesis of group A streptococcal infections, and post-infection sequelae, will be difficult to assess.

The purpose of this study was to isolate and characterize the type II Fc receptors found on certain group A streptococci and to examine the distribution of the type II Fc receptors and their relationship in the pathogenesis of streptococcal diseases. The specific aims were to:

1. Develop a method to screen bacterial isolates for Fc receptor proteins, in particular the type II Fc receptors (Chapter Two).
2. Isolate and characterize the type II Fc receptors from group A streptococci (Chapter Three).
3. Determine the distribution of the type II Fc receptors on nephritogenic and non-nephritogenic group A streptococci (Chapter Four).

CHAPTER TWO
A TECHNIQUE FOR THE DETECTION OF BOUND AND SECRETED BACTERIAL Fc RECEPTORS

Introduction

The type II Fc receptor on certain group A streptococci has proved difficult to study because it is only found at low levels on positive strains and is frequently lost during subculturing. The initial aim of this study was, therefore, to establish a rapid method to screen group A streptococcal strains for Fc receptors and to identify and expand individual colonies that expressed high levels of these receptors.

Previous studies have shown that passage of streptococci in mice resulted in enhanced Fc receptor expression (Burova et al., 1980, 1981). This approach has been used to isolate a group A streptococcal strain with increased Fc receptor expression (Reis et al., 1984d). Until a method is developed to screen fresh isolates of group A streptococci without subculturing, it will be difficult, however, to assess the role that this receptor may play in group A streptococcal infections and post-infection sequelae.

This chapter describes a semi-quantitative procedure that enables isolates of group A streptococci to be screened rapidly for Fc receptor expression. Using this technique, a mouse-passaged group A streptococcal strain that was selected for its high levels of surface Fc receptors (Reis et al., 1984d) was shown to be heterogeneous in the level of expression among individual colonies. Colonies expressing high levels of surface Fc receptors were selected from replica plates

and were shown to maintain a higher average level of Fc receptor expression on repeated subculture.

Materials and Methods

Bacterial Strains, Media, and Growth Conditions

Laboratory strains and fresh isolates of β-hemolytic streptococci, obtained from Dr. Elia Ayoub at the University of Florida, College of Medicine, and Staphylococcus aureus strains, obtained from the American Type Culture Collection, were used in these studies. β-hemolytic streptococci were grouped by the Phadebact Streptococcus Test, Pharmacia Diagnostics, Piscataway, NJ. All strains were grown in Todd-Hewitt broth (DIFCO) for 18-24 hr at 37°C, harvested by centrifugation, and washed in phosphate-buffered saline (PBS), pH 7.2. The optical density at 550 mm was determined to standardize the concentration of organisms used in subsequent tests.

Staphylococcus aureus Cowan I and Staphylococcus aureus Wood 46 served respectively as high and low protein A (PA)-producing strains.

IgG and F(ab')$_2$ Fragments

Stock human IgG was prepared by chromatography of normal human serum on DEAE cellulose (Boyle and Langone, 1980). Aliquots were stored at -70°C until use. Human IgG F(ab')$_2$ fragments were prepared by pepsin digestion of the stock IgG preparation by the method described by Reis et al. (1983). The IgG and F(ab')$_2$ fragments were prepared from an individual donor and therefore the IgG and F(ab')$_2$ fragments displayed the same distribution of antigenic reactive antibodies.

Iodination of IgG

Purified human IgG was iodinated by mild lactoperoxidase method using enzyme beads (Bio-Rad) as described previously (Reis et al., 1983). The IgG routinely had a specific activity of 0.3 mCi/mg.

Detection of Surface Bacterial Fc Receptors

An overnight suspension of bacteria was diluted in Todd-Hewitt broth to give 10-100 colonies when 0.1 ml was plated on Todd-Hewitt agar (Todd-Hewitt broth containing 1.5% agar). The plates were incubated at 37°C for 16 h and replica plated onto blood agar plates (BBL Microbiology Systems, Cockeysville, MD 21030) as described by Lederberg and Lederberg (1952). A circular piece of nitrocellulose, previously soaked in 25 mM Tris, 192 mM glycine (pH 8.3) and 20% v/v methanol followed by a circular piece of Whatman 3 mm paper was placed on top of the colonies. The agar was removed from the petri dish and a piece of 3 mm paper was placed on the bottom of the agar. The bacterial colonies were transferred to the nitrocellulose by electrophoresis at 70 V for 3 h in the above buffer. This procedure resulted in quantitative transfer of the bacterial colonies, as judged by comparing photographs of plates before transfer with the stained nitrocellulose membrane after the electroblotting procedure.

After electrophoresis, the nitrocellulose was washed in veronal buffered saline (VBS) containing 0.25% gelatin and 0.25% Tween-20 for one hour with four 250 ml changes. The nitrocellulose was probed for 3 h in the washing buffer containing 2 x 10^5 cpm/ml ^{125}I-labeled human IgG and a two-fold excess of unlabeled $F(ab')_2$ fragments. Inclusion of $F(ab')_2$ fragments in the probing mixture eliminates any binding of IgG through antigenic recognition sites (Reis et al., 1983). After probing, the nitrocellulose was washed four times in 0.01 M EDTA, 1 M NaCl, 0.25% gelatin, and 0.25% Tween-20 for 15 min each and allowed to air dry. The nitrocellulose blots were autoradiographed by exposing

to Kodak XAR-5 film with intensifying screen for 3 days at -70°C. This procedure is summarized in Figure 2-1.

Absorption of ^{125}I-Labeled IgG by Bacteria

The detection of Fc-reactive proteins on the surface of bacteria was determined by the ability of bacteria to bind ^{125}I-labeled human IgG using a modification of the method of Myhre and Kronvall (1977). Briefly, a standard number of bacteria was incubated with 20,000 cpm ^{125}I-labeled human IgG and a twofold excess of unlabeled $F(ab')_2$ fragments for 1.5 h at 37°C. The bacteria were pelleted by centrifugation at 1000 x g for 5 min and washed twice with 2 ml of VBS containing 0.01 M EDTA and 0.1% gelatin (EDTA-gel). The radioactivity associated with the bacteria was determined in an LKB autogamma counter. The cpm bound to the bacterial pellet were expressed as percent of added radioactivity.

Results

Fc Receptor Expression of Staphylococcus aureus

The purpose of this study was to develop a procedure that could rapidly screen isolated colonies of bacteria for Fc receptor expression. Initially I used the protein A-rich Staphylococcus aureus Cowan I strain as a reference high level positive control and the Staphylococcus aureus Wood 46 strain as a reference low level positive control. In these experiments, a dilution of Staphylococcus aureus Cowan I or Wood 46 strain was seeded onto Todd-Hewitt agar plates to yield approximately 50 colonies following overnight incubation at 37°C. The colonies were transferred to nitrocellulose and probed with ^{125}I-labeled human IgG containing a two-fold molar excess of

unlabeled $F(ab)_2$ fragments. The $F(ab')_2$ fragments were prepared from the same source of human IgG as that used to prepare the labeled tracer. Previous studies have shown that inclusion of the $F(ab')_2$ fragments in the probing mixture eliminates any binding of IgG through antigenic recognition sites (Reis et al., 1983). Consequently, only binding via the Fc region is measured using this probing mixture. The procedure is detailed in the Methods and summarized in Figure 2-1. The results of a typical experiment using Staphylococcus aureus strains are presented in Figure 2-2. The Staphylococcus aureus Cowan I strain demonstrated a greater expression of Fc receptors than Wood 46 strain. The surface expression of Fc receptors on an individual plate was consistent from colony to colony, for both the Cowan I and the Wood 46 strain.

Quantification of Secreted Fc Receptors

Secreted Fc receptors were measured using a modification of the blotting procedure described in Figure 2-1. After removal of the agar from the petri dish, the nitrocellulose was placed on the underside of the agar away from the bacteria rather than directly in contact with the colonies. Fc receptors that were secreted by the bacteria were electroblotted through the agar and bound to the nitrocellulose while the colonies were unable to pass through the agar. The nitrocellulose was probed as before with ^{125}I human IgG containing unlabeled $F(ab')_2$ fragments, and then autoradiographed. Figure 2-2 illustrated the secretion of Fc receptors by the Cowan I and Wood 46 strain. As expected, the Cowan I strain secreted high levels of Fc receptor activity. By contrast, the Wood 46 strain did not secrete sufficient protein A to be detected by this procedure, see Figure 2-2.

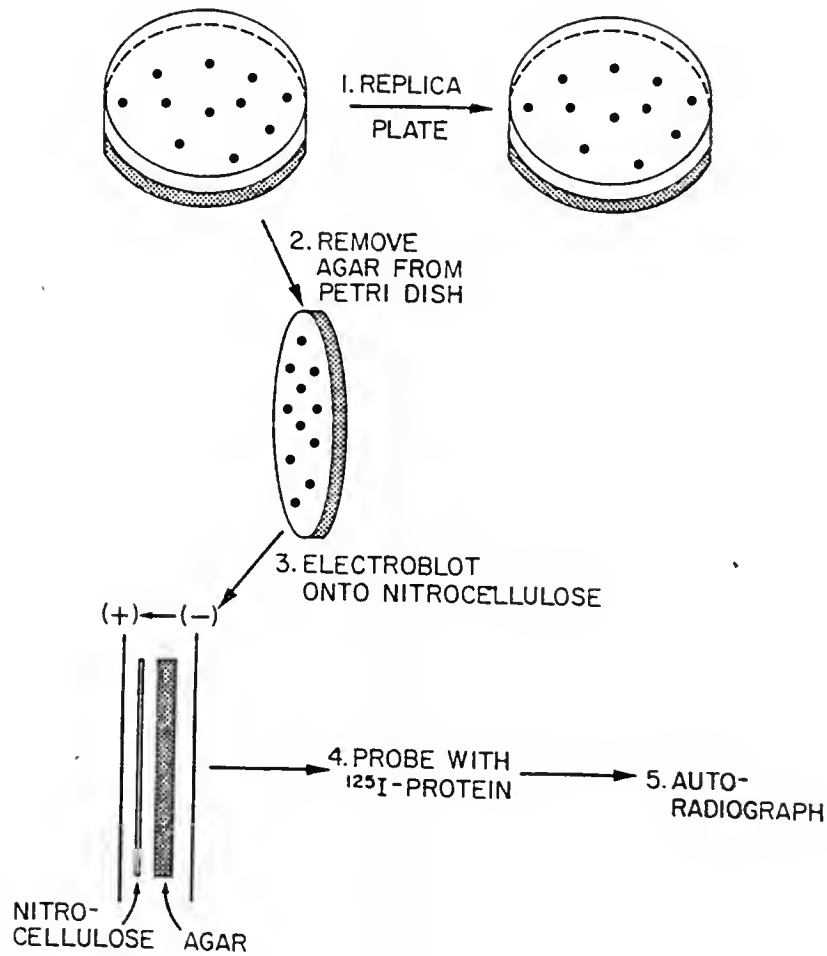


Fig. 2-1. Schematic representation of the immunoblotting procedure for detection of surface Fc receptors.

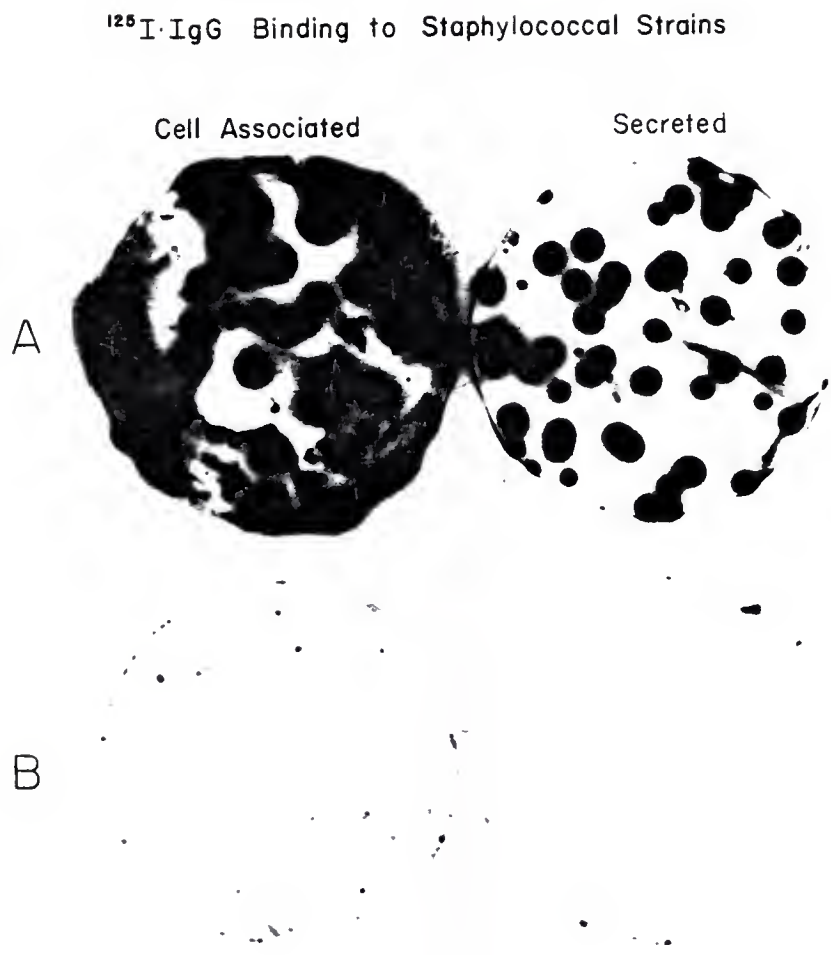


Fig. 2-2. Detection of Fc receptors on the surface of staphylococcal strains.

A, Staphylococcus aureus Cowan I strain; B, Staphylococcus aureus Wood strain. The colonies of staphylococci were blotted onto nitrocellulose and probed with ^{125}I-labeled human IgG in the presence of unlabeled $F(ab')_2$ fragments as described in the text. Autoradiography was carried out by exposure of the blot for 3 days at -70°C to X-ray film using an intensifying screen.

In order to determine the sensitivity of this method for detecting secreted Fc receptors, differing concentrations of purified protein A (0.05-500 ng/5 µl) were applied directly to agar plates. The agar was removed and treated as described above. The applied protein A was electroblotted through the agar onto nitrocellulose and probed for binding of labeled ^{125}I human IgG. The blot was autoradiographed at -70°C for one day and the diameter of the resulting dot was measured. The area of the dot was proportional to the square of its radius (r). When the value of r^2 for each concentration of protein A originally applied to the agar was plotted against the concentration of protein A added, a linear relationship was observed over the range 6.25 to 50 ng, see Figure 2-3.

Fc Receptor Expression on a Group A Streptococcal Strain

In the initial experiments a mouse-passed strain of group A streptococci, 64/14, which has been shown to have high levels of surface Fc receptor (Reis et al., 1984d), was selected to study the expression of Fc receptors on the surface of streptococci. A single colony of the mouse-passed strain 64/14 was grown overnight in Todd-Hewitt Broth. Bacteria were then diluted as described in the Methods to yield 10-100 colonies per plate and tested for Fc receptor expression. The Fc receptor expression on the individual colonies of strain 64/14 is illustrated in Figure 2-4. Unlike the uniform pattern observed with Staphylococcus aureus strains (Figure 2-2), the intensity of the spots on the autoradiograph varied considerably, indicating heterogeneity in expression of Fc receptors on individual colonies. This effect was not due to variation in the size of individual colonies, since, as shown on the replica plated sample, the colony size

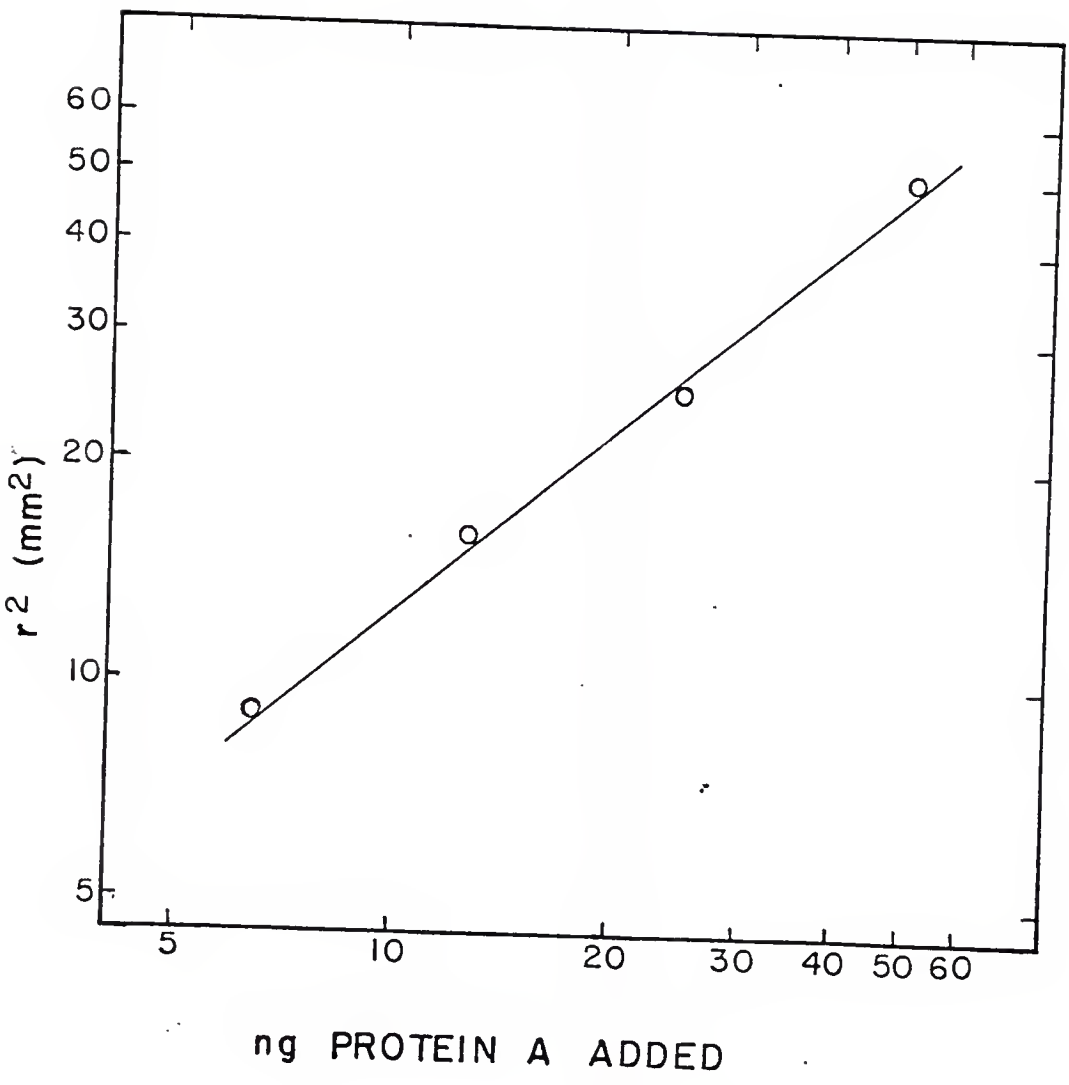


Fig. 2-3. Sensitivity of assay for secreted Fc receptors.
Protein A from 0.05 to 500 ng was applied in 5 µl of VBS, to a culture plate, electroblotted through the agar onto nitrocellulose and then probed with ^{125}I-labeled human IgG in the presence of unlabeled $F(ab')_2$ fragments. The blot was autoradiographed by exposure for 1 day at -70°C to X-ray film using an intensifying screen and the diameter of the resulting dot on the X-ray film was measured. The value of r^2, which is proportional to the area of the dot, was plotted against the concentration of protein A applied to the plate.

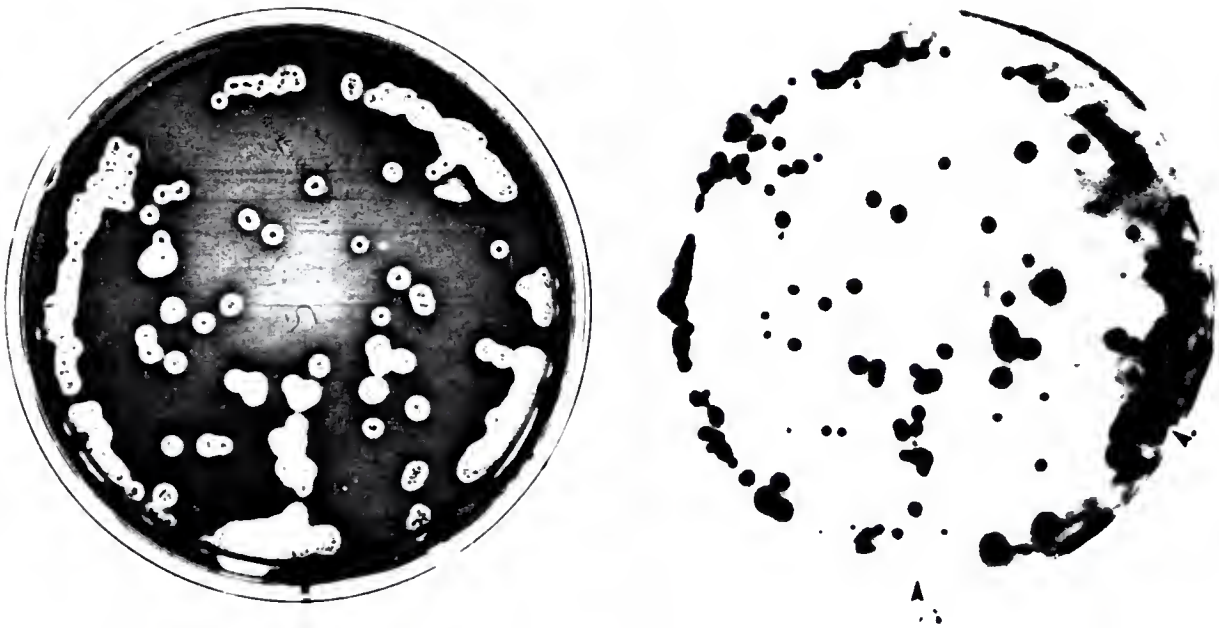


Fig. 2-4. Fc receptor expression of a group A streptococcal strain.
The panel on the left of the figure shows colonies of strain 64/14 on a blood agar replica plate. The panel on the right is an autoradiograph of a replica plate following the probing with ^{125}I-labeled human IgG in the presence of unlabeled $F(ab')_2$ fragments. Autoradiography was carried out by exposure of the blot for 3 days at -70°C to X-ray film using an intensifying screen.

was remarkably uniform. A representative high intensity (Fc receptor-rich) and low intensity (Fc receptor-poor) colony was selected from the replica plate and subcultured. The expression of surface Fc receptor on the progeny of a low-producer colony and a high-producer colony are shown in Figure 2-5. The progeny of the high producing colony maintained greater levels of Fc receptor expression than the progeny of the low producing colonies; however, heterogeneity in surface expression of Fc receptors within the selected strains was still observed. On repeated subculturing, however, the low and high producers remained readily distinguishable, and none of these strains secreted measurable quantities of the Fc receptor.

The differences in average Fc receptor expression of these two bacterial subpopulations were also tested in a direct binding assay. In this assay the indicated number of bacteria was incubated at 37°C for 1.5 h with 2×10^4 cpm/0.1 ml ^{125}I human IgG and a two-fold molar excess of unlabeled $F(ab')_2$ fragments in a total volume of 0.2 ml VBS-gel buffer. The bacteria were pelleted by centrifugation at 1000 x g for 5 min and washed twice with 2 ml of EDTA-gel buffer and the number of cpm bound to the bacteria determined. The results in Figure 2-6 demonstrate greater IgG binding to the high producing population than to the low producing population, with the parental 64/14 strain binding an intermediate level. These results are in agreement with the findings in Figure 2-5 and would suggest that the blotting assay is accurately reflecting surface Fc receptor expression on these bacteria.

Fig. 2-5. Fc receptor expression of two bacterial subpopulations selected from the original strain 64/14.

The autoradiograph in the left panel was obtained by probing a high Fc receptor producing substrain and the autoradiograph in the right panel was obtained by probing a low Fc receptor-producing colony. These colonies were selected and subcultured from individual colonies in the replica plate shown in Figure 2-4. Autoradiography was carried out by exposure of the blot for 3 days at -70°C to X-ray film using an intensifying screen.

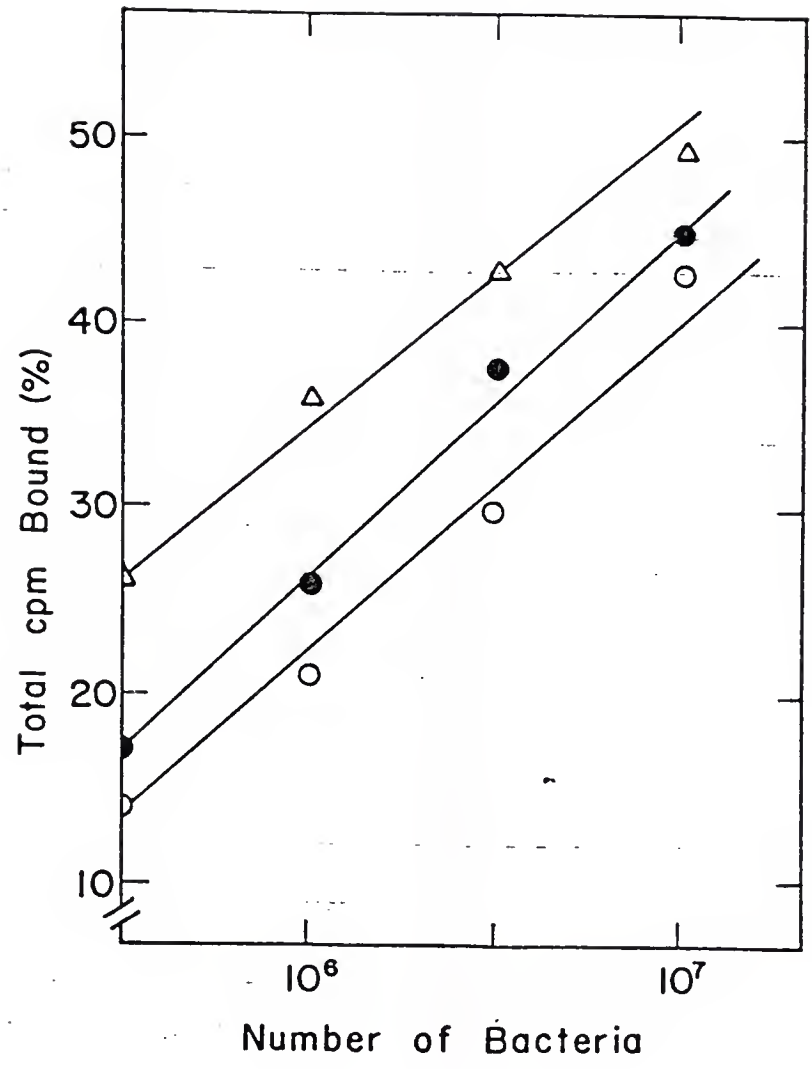


Fig. 2-6. Fc dependent uptake of ^{125}I-labeled human IgG by increasing numbers of bacteria. ●—●, parental strain 64/14; △—△, high Fc receptor producing substrain from 64/14; ○—○, low Fc receptor producing substrain from 64/14.

Discussion

In this chapter, a method for rapidly screening fresh isolates of streptococci for Fc receptors has been described. This technique can measure both cell-associated and secreted Fc receptors and is capable of identifying individual colonies that express high levels of these receptors. Initially, the method was developed using Fc receptor positive Staphylococcus aureus strains that express stable levels of the type I Fc receptor on their surface. These were the protein A-rich Cowan I strain and the protein A-poor Wood 46 strain (Freimer et al., 1979; Reis et al., 1984c). The conditions for electroblotting bacterial colonies onto nitrocellulose and probing for Fc receptor expression were established using these strains (Fig. 2-2). The ^{125}I-labeled human IgG probe was made Fc receptor-specific by including a two-fold molar excess of unlabeled $F(ab')_2$ fragments from the same IgG pool used to prepare the labeled tracer. Under these conditions the unlabeled $F(ab')_2$ fragments inhibit the binding of any specific anti-bacterial antibody (Reis et al., 1983). In agreement with previous reports, all of the staphylococcal colonies expressed surface protein A, and there was a marked quantitative difference between the levels expressed on the Cowan and Wood strains (Freimer et al., 1979; Reis et al., 1984c).

When this approach was applied to a mouse-passaged group A streptococcal strain, the intensity of individual autoradiographed streptococcal colonies showed wide variation (Fig. 2-4). In order to ensure that these findings were reproducible, one colony that expressed high levels of Fc receptors and a second colony, with a low level of Fc receptor expression, were subcultured from the replica plate.

Following subculture, each colony was plated, electroblotted and probed as described. The results indicated that the intensity of the progeny colonies were markedly different (Fig. 2-5). Differences in surface Fc receptor expression of the two substrains were confirmed by direct Fc-mediated binding of labeled human IgG (Fig. 2-6). In the two days required to expand a high or low expressing population, considerable heterogeneity in Fc receptor expression on the resulting colonies was readily detected (Fig. 2-4). These findings suggest that the expression of these type II Fc receptors on group A streptococci is constantly shifting and could explain many of the reports of changes in, or loss of, Fc receptor expression during subculture of group A streptococcal strains (Kronvall, 1973a; Christensen and Oxelius, 1974; Freimer et al., 1979).

The presence of extrachromosomal DNA in certain group A streptococci has been reported (Clewell, 1981; Burova et al., 1983; Ravdonikas, 1983; Ravdonikas et al., 1984). Conjugation experiments between group A streptococcal strains with Fc receptors and group A streptococcal strains without Fc receptors, suggest that either Fc receptor expression is controlled by a plasmid, or that the gene for Fc receptors is encoded on a plasmid (Burova et al., 1983; Ravdonikas et al., 1984). No plasmids have been isolated, however, from the mouse-passaged group A streptococcal strain 64/14 (data not shown). The possibility still exists that the plasmid has integrated into the genome, but more studies are needed in order to understand the control of Fc receptor expression that is expressed on group A streptococcal strains.

In addition to providing a rapid, semi-quantitative assay for surface Fc receptor expression on individual colonies of bacteria, the method described was readily modified to measure secreted Fc receptors. This was achieved by carrying out the electroblotting stage of this assay with the nitrocellulose placed on the opposite side of the agar from the bacterial colonies. The approach readily detected nanogram quantities of secreted protein A from the staphylococcal strains (Fig. 2-3). To date, no Fc receptor secreting group A streptococcal strain has been identified.

The method described in this chapter for screening individual colonies of bacteria for Fc receptors has enabled me to monitor type II Fc receptor expression on a group A streptococcal strain. By continual selection, I can now maintain a strain rich in the type II receptor from which to isolate this receptor.

This method can also be used to screen bacteria for other receptors. Using a modification of the procedure β_2-microglobulin, fibrinogen, fibronectin, and collagen type I and type III receptors were found on certain isolates of staphylococci and streptococci (see Chapter Four). In addition, this method can be applied to the study of fresh isolates of bacteria, to determine if the presence or absence of a particular protein, or receptor, correlates with subsequent disease course.

CHAPTER THREE
ISOLATION AND PARTIAL CHARACTERIZATION OF THE TYPE II Fc RECEPTORS FROM A GROUP A STREPTOCOCCUS

Introduction

Although many group A streptococci have surface receptors for the Fc region of all four subclasses of human IgG (Myhre and Kronvall, 1977,1979,1980b,1981; Reis *et al.*, 1984d), these receptors are frequently lost during subculturing (Christensen and Oxelius, 1974; Freimer *et al.*, 1979), making the isolation and characterization of these proteins difficult. Recently Reis *et al.* (1984d) have described the isolation of a stable Fc receptor-rich group A streptococcal strain. This strain, which was recovered following fourteen sequential passages of a group A streptococcus in mice (Reis *et al.*, 1984d), demonstrated Fc receptor expression approaching that of the protein A rich *Staphylcoccus aureus* Cowan I strain (Reis *et al.*, 1984d). Although it has maintained a high level of Fc receptor expression for over two years, heterogeneity of expression was observed between individual colonies when monitored using an immunoblotting assay (Chapter Two). The immunoblotting technique has enabled me to monitor Fc receptor expression continuously on individual colonies and to select substrains with high levels of Fc receptor expression. In this Chapter, I describe the isolation and characterization of the Fc receptors from such an Fc receptor-rich group A streptococcal substrain.

Materials and Methods

Bacterial Strains, Media, and Growth Conditions

The mouse-passaged group A streptococcal strain 64/14 was shown in Chapter Two to contain colonies with different levels of surface expression of Fc receptors. Using the immunoblotting technique described in Chapter Two, a single colony expressing high surface Fc receptor activity (64/14/HRP) was selected and used as the source for the isolation of the type II Fc receptor. Staphylococcus aureus Cowan I strain served as a representative type I Fc receptor-positive strain and the group C streptococcal strain 26RP66 served as a type III receptor-positive strain. All strains were grown in Todd-Hewitt broth (DIFCO) as stationary cultures for 18-24 hr at 37°C, harvested by centrifugation and resuspended in phosphate-buffered saline (PBS), pH 7.2. The optical density at 550 nm was determined to standardize the concentration of organisms used in subsequent tests. An OD_{550} of 0.3 corresponded to approximately 2×10^9 organisms/ml.

Dipeptides

Glycyl-L-tyrosine, glycyl-L-histidine, and glycylglycine were purchased from Sigma Chemical Company, St. Louis, MO.

Extraction of Fc Receptors

The selected group A streptococcal strain 64/14/HRP was grown overnight at 37°C in Todd-Hewitt broth. Approximately 6 g (wet weight) of bacteria were recovered from 3 liters of Todd-Hewitt broth.

Mutanolysin extraction. Approximately 6 g (wet weight) of group A strain 64/14/HRP were suspended in 30 ml of 20 mM Tris-HCl, pH 7.5, 1 mM iodoacetic acid, and 1 mM benzamidine HCl. To this suspension, 100 µg/ml pancreatic DNase (Sigma), 100 µg/ml pancreatic RNase (Sigma), and

100 µg/ml mutanolysin were added. A commercial mutanolysin preparation (Sigma) was further purified to remove protease activity using the method described by Siegel et al. (1981). The enzyme and bacteria were incubated at 37°C in a shaking water bath for 4 hr. The mixture was then centrifuged at 10,000 g for 10 min and the resulting supernatant filtered through a 0.2 µm filter to remove the remaining bacteria. The filtrate was dialyzed against 20 mM Tris-HCl, pH 7.5, containing 1 mM iodoacetic acid, 1 mM benzamidine-HCl, and 1 mM phenylmethyl sulfonyl fluoride (PMSF).

Hyaluronidase extraction. Approximately six grams (wet weight) of the group A strain, 64/14/HRP, were suspended in 30 ml of 0.15 M PBS, pH 7.2. To this suspension, 10 mg type IV hyaluronidase (Sigma) was added and incubated at room temperature for 30 min. The bacteria-free supernatant was recovered as described above.

Papain digestion. Group A strain 64/14/HRP, approximately 2 g (wet weight), was suspended in 20 mls of 10 mM Tris-HCl, pH 8.0 and 0.02% NaN_3. Two milliliters of 0.4 M cysteine and 1.6 mg papain were added to this suspension and allowed to incubate at 37°C for 1 hr. The reaction was stopped by addition of iodoacetic acid to a final concentration of 30 mM. The bacteria-free supernatant was recovered as described above.

Trypsin digestion. Approximately 2 grams (wet weight) of group A strain 64/14/HRP in 50 mM KPO_4, 5 mM EDTA, 0.02% NaN_3, pH 6.1 (20 ml) was incubated at 37°C for 1 hr. with 80 µg pancreatic DNase and 400 µg trypsin (Sigma). Addition of benzamidine-HCl to a final concentration of 100 mM stopped the reaction. Bacteria were removed as described above.

Phage lysin treatment. Approximately 2 g (wet weight) of the group A strain 64/14/HRP were suspended in 20 ml of 50 mM KPO_4, 5 mM EDTA, 0.02% NaN_3, pH6.1. Phage lysin (0.2 ml), previously activated by incubation at room temperature for 15 min. in dithiothreitol (DTT) at a final concentration of 50 mM, was added to the 10% suspension and incubated at 37°C for 1 hr. The phage lysin was prepared as previously described by Fischetti et al. (1971). The bacteria-free supernatant was recovered as described above.

SDS treatment. A 10% (w/v) suspension of group A strain 64/14/HRP in 1% sodium dodecyl sulfate was incubated at 80°C for 10 min. Bacteria were removed as described above and SDS was removed by dialysis against 20 mM Tris-HCl, pH 7.5.

Autoclave treatment. A 10% (w/v) suspension of group A strain 64/14/HRP was autoclaved at 124°C for 15 min, with or without the addition of 1% SDS.

Hot acid/hot alkaline extractions. Extractions were carried out according to the method of Lancefield (1928). Bacteria were suspended in 0.15 M PBS to form a 10% suspension and the pH was adjusted to 2 (or 10) with 0.5 M HCl (or 0.5 M NaOH). The bacterial suspension was boiled for 10 min and the pH neutralized. The bacteria-free supernatants were recovered as described previously.

Heat extraction. This was carried out as described above, but at neutral pH using PBS, pH 7.0.

IgG and IgG Subclasses

Stock human IgG was prepared by chromatography of normal human serum on DEAE cellulose (Boyle and Langone, 1980). Aliquots were stored at -70°C until use. Human IgG_3 (κ) cryoglobulin was a gift

from Dr. Richard Weber, National Institutes of Health, Bethesda, MD. The cryoglobulin was isolated as described in (Saluk and Clem, 1971). Human IgG subclasses were provided by the WHO/IUIS Immunoglobulin Subcommittee.

IgG_1 (κ) Lot No. 0781 and IgG_1 (λ) Lot No. 0180;

IgG_2 (κ) Lot No. 0380 and IgG_2 (λ) Lot No. 0981;

IgG_3 (κ) Lot No. 0282 and IgG_3 (κ) Lot No. 0784 and

IgG_3 (λ) Lot No. 0381;

IgG_4 (κ) Lot No. 0981 and IgG_4 (λ) Lot No. 0880

Purified rabbit, cow, sheep, goat, rat, dog, and pig IgG was purchased from Cappel Laboratories, Inc., Cochranville, PA.

Immobilized IgG Preparations

Antigens were coupled to immunobeads (Bio-Rad) by the method described in Reis et al. (1983). The ligand to be immobilized was covalently bound to the immunobead matrix by peptide bond formation between the carboxylic groups on the beads and amino acids groups of the ligand. This reaction is catalyzed by carbodiimide and the resulting beads can be stored for up to six months at 4°C in the presence of 0.02% sodium azide without loss of reactivity.

Immobilized IgG for affinity purification of the streptococcal Fc receptor was prepared by covalently coupling human IgG to the high capacity Affi-gel 15 activated beads (Bio-Rad) as described in (Reis et al., 1984b).

Iodination of IgG and Protein A

Purified Protein A (Pharmacia, Piscataway, NJ) or purified IgG or IgG subclasses were iodinated by mild lactoperoxidase method using enzyme beads (Bio-Rad) as described previously (Reis et al., 1983). The IgG or protein A routinely had a specific activity of 0.3 mCi/mg.

Preparation of Fc Specific Probe

^{125}I labeled human IgG was made Fc specific by the inclusion of a twofold molar excess of unlabeled $F(ab')_2$ fragments in the probing mixture (Reis et al., 1983). Human IgG $F(ab')_2$ fragments were prepared by pepsin digestion of the stock IgG preparation by the method described by Reis et al. (1983). The IgG and $F(ab')_2$ fragments were prepared from an individual donor and consequently the IgG and $F(ab')_2$ fragments contained the same distribution of antigenic reactive antibodies. Therefore, only binding via the Fc region is measured using this probing mixture.

Competitive Binding Assay For Soluble Bacterial Fc Receptor

Fc receptor activity in extracts were quantified using the competitive binding assay of Reis et al. (1983). This assay was carried out using VBS-gel as the diluent. In this assay 1.0 ml of a test sample or buffer was mixed with 0.1 ml of a standard suspension of agarose beads with covalently coupled human IgG (Bio Rad Laboratories, Richmond, CA), and 0.1 ml of ^{125}I protein A (approximately 20,000 cpm) and incubated at 37°C for 90 min. Two milliliters of veronal buffered saline containing 0.01 M trisodium ethylenediamine tetra-acetate and 0.1% gelatin (EDTA-gel) was added to each tube and centrifuged at 1,000 g for 5 minutes and the supernatant fluid decanted. After an additional wash, the radioactivity associated with the beads was determined in an LKB Gamma Counter.

Gel Electrophoresis and Western Blotting

Proteins were analyzed by electrophoresis under denaturing conditions in polyacrylamide gels containing sodium dodecyl sulfate according to Laemmli (1970). Gels for staining were fixed in a

solution of 40% ethanol and 10% acetic acid, stained with Coomassie brilliant blue R-250 (0.25% w/v in 40% ethanol and 10% acetic acid) for 1 hr, and destained by soaking in several changes of 10% ethanol and 10% acetic acid. Gels used for blotting were equilibrated in 25 mM Tris, 192 mM glycine, pH 8.3 and 20% v/v methanol (transfer buffer) for 30 min. A piece of nitrocellulose, previously soaked in the transfer buffer, was placed on top of the gel. The gel and nitrocellulose were sandwiched between 2 pieces of Whatman 3 mm paper and placed in a Bio-Rad Trans Blot apparatus with the nitrocellulose oriented between the anode and the gel. Electrophoresis was at 70 volts for 3 hr in the above buffer.

After electrophoresis, the nitrocellulose was washed in veronal buffered saline (VBS) containing 0.25% gelatin and 0.25% Tween-20 for 1 hr with four 250 ml changes. The nitrocellulose was probed for 3 hr in the washing buffer containing 2×10^5 cpm/ml of the appropriate species or subclass of IgG. After probing the nitrocellulose was washed four times in 0.01 M EDTA, 1 M NaCl, 0.25% gelatin, and 0.25% Tween-20 for 15 min each and allowed to air dry. The nitrocellulose blots were autoradiographed by exposing to Kodak XAR-5 film with intensifying screen for 1 to 3 days at -70°C.

Dot-Blot Procedure to Test Species Reactivity

Dot blots were performed using the Bio-Rad bio-dot microfiltration apparatus and a modification of the Bio-Rad procedure. A standard number of group A strain 64/16/HRP was incubated at 37° for 1 hr with increasing concentrations of dog, goat, pig, sheep, rabbit, rat, bovine, or human IgG. A piece of nitrocellulose, previously soaked in 25 mM Tris, 192 mM glycine, pH 8.3, and 20% V/V methanol (wash buffer),

was placed in the apparatus. Following incubation, the mixture was pipetted into the wells and washed with the above buffer. The nitrocellulose was removed from the apparatus, washed and probed as described above in the Western blotting procedure.

Preparation of Monospecific Antibodies to a Single Species of Affinity Purified Fc-Reactive Material

Monospecific antibodies were prepared in chickens whose non-immune IgG does not react with the Fc-receptor protein being studied. The choice of a non-reactive host to immunize is important to avoid complications with hypersensitivity and Arthus reactions (Gustafson et al., 1968). Immunoglobulins were isolated from egg yolks as described below. Eggs from a white Leghorn chicken were collected prior to injection with isolated Fc-reactive protein. This provided a source of pre-immunization IgG from an individual animal. This was used as a control for later studies. The chicken was then injected with an immunogen containing approximately 50 µg of Fc-reactive material intramuscularly or subcutanously in complete Freund's adjuvant. The immunogen used was a single form of the Fc-receptor protein that was isolated first by affinity purification by binding to, and elution from a column of immobilized IgG, and then further purified by SDS polyacrylamide gel electrophoresis. The gel was stained with Coomassie blue, and a single stained band was cut from the gel, emmulsified in adjuvant and used as the immunogen. The chickens were allowed to rest for three weeks and then injected with approximately 50 µg of the immunogen prepared as described above that had been emmulsified in incomplete Freund's adjuvant. Eggs were collected from the chickens, immunoglobulins were extracted, and the production of antibody was monitored as described below.

Chloroform Extraction of Egg Yolks

Eggs from the chicken were extracted using a modification of the procedure described by Aulisio and Shelokov (1967). Briefly, the yolks were separated from the albumin and adhering membrane, diluted with an equal volume of PBS, and shaken several times. The suspension stood at room temperature for 10 min. The extraction was repeated a total of 4 times before centrifugation at 10,000 g for 20 min. To the supernatant, an equal volume of chloroform was added and the mixture was shaken at room temperature every 30 min for 2 hr before incubating at 4°C overnight. The extraction was centrifuged at 5,000 g for 10 min. The resulting clear supernantant was assayed for antibody production by measuring the inhibition of ^{125}I labeled human IgG binding to the type II receptor-rich group A bacterial strain, 64/14/HRP.

Chicken Anti-Type I and Anti-Type III Antibodies

Monospecific antiserum to the staphylococcal type I Fc receptor was prepared as described in (Reis et al., 1984c). Monospecific antiserum to the streptococcal type III Fc receptor was prepared as described by Reis et al. (1984a).

Immobilized Anti-Type II Fc Receptor

Antibodies raised against the affinity purified 38,000 dalton (type IIb) Fc receptor present in the affinity purified heat extract of 64/14/HRP was prepared as described above. This anti-type IIb Fc receptor antibody was isolated by chloroform extraction of the yolks of eggs from immunized chickens. The antibodies were concentrated by ammonium sulfate precipitation (40%) and covalently coupled to Affi-gel 15 activated beads (Bio-Rad) as described in (Reis et al., 1984a). The immobilized anti-type IIb Fc receptor was used for the affinity purification of the type II Fc receptors.

Purified Type I and Type III Fc Receptors

Purified type I Fc receptor (protein A) was obtained from Pharmacia Fine Chemicals, Piscataway, NJ. The type III Fc receptor was purified as described by Reis, et al. (1985).

Results

Solubilization of The Type II Fc Receptor

A mouse-passaged group A streptococcal strain, 64/14/HRP, was selected because of its high levels of surface Fc receptors (Chapter Two) and its stability on subculture (Reis et al., 1984d). Several extraction procedures were compared, including: 1) hot-acid extraction, alkaline extraction, or heat extraction at neutral pH; 2) treatment with the enzymes hyaluronidase, mutanolysin, papain, trypsin, or phage lysin; or 3) heating, or autoclaving the bacteria in the presence of sodium dodecyl sulfate (SDS). The resulting cell-free extracts were tested for soluble Fc receptor activity using two assays. The first was a competitive binding assay, that measures the inhibition of ^{125}I labeled protein A (the type I Fc receptor) to immobilized human IgG and is capable of detecting nanogram quantities of Fc-reactive proteins (Reis et al., 1983). The second assay was a Western blotting procedure. In this technique, the extractions were electrophoresed on an SDS polyacrylamide gel, electroblotted onto nitrocellulose, and probed with the ^{125}I labeled human IgG Fc specific probe. By running a duplicate gel and staining with Coomassie brilliant blue, Fc receptor activity can be matched to specific protein bands. The Western blotting procedure has a number of advantages. First, it enabled size heterogeneity of Fc receptors to be detected and second, it detected Fc reactivities with sites on IgG not related to

the binding site for staphylococcal protein A. Details of these procedures are described in the Materials and Methods.

The only treatments that resulted in the solublization of significant quantities of Fc receptor activity were heat extraction at neutral pH, and treatment with the enzymes mutanolysin or hyaluronidase (Table 3-1). Extraction with SDS, or autoclaving in the presence of SDS, also resulted in solubilizing Fc receptor activity, but with lower specific activity. Extraction of the group A streptococci with acid, alkali, papain, trypsin, phage lysin, or by autoclaving did not solubilize detectable quantities of a functionally active Fc receptor in either assay. Comparison of the extracts by Western blot analysis showed that the extract with the least heterogeneity resulted from heating the bacteria at neutral pH (Fig 3-1). This extraction procedure was consequently used to isolate the type II Fc receptor.

Isolation of the Type II Fc Receptor

The type II Fc receptor-rich, group A streptococcus was heat extracted as described in the Methods. The bacteria-free extract was dialyzed against 20 mM Tris-HCl, pH 7.5 containing protease inhibitors at a concentration of 1 mM phenylmethyl sulfonyl fluoride (PMSF), 1 mM iodoacetic acid, and 1 mM benzamidine-HCl to prevent degradation of the Fc receptors (Grubb et al., 1982).

The dialyzed extract was applied to a column of human IgG immobilized on Affi-gel 15 which had been prewashed with 3 M $MgCl_2$ and then equilibrated in 20 mM Tris-HCl, pH 7.5, containing protease inhibitors. The extract was applied to the column, and unbound material was eluted by washing with the Tris-HCl buffer. Fc receptor was eluted by addition of 3 M $MgCl_2$ to the 20mM Tris-HCl, pH 7.5

TABLE 3-1
Fc Receptor Activity in Streptococcal Extracts

Extraction	Yield (μg/g bacteria)	Western Blot Analysis (Number of protein bands)	Competitive Binding Assay (μg required for 50% inhibiton)
Heat	36	2	1.5
Hot acid	--	None	No inhibition
Hot alkaline	--	None	No inhibition
Hyaluronidase	24	4	8.4
Mutanolysin	115	>8	0.2
Papain	--	None	No inhibition
Trypsin	--	None	No inhibition
SDS	--	7	>200
Autoclaving	--	None	No inhibition
Autoclaving + SDS	--	5	>200
Phage lysin	--	None	No inhibition

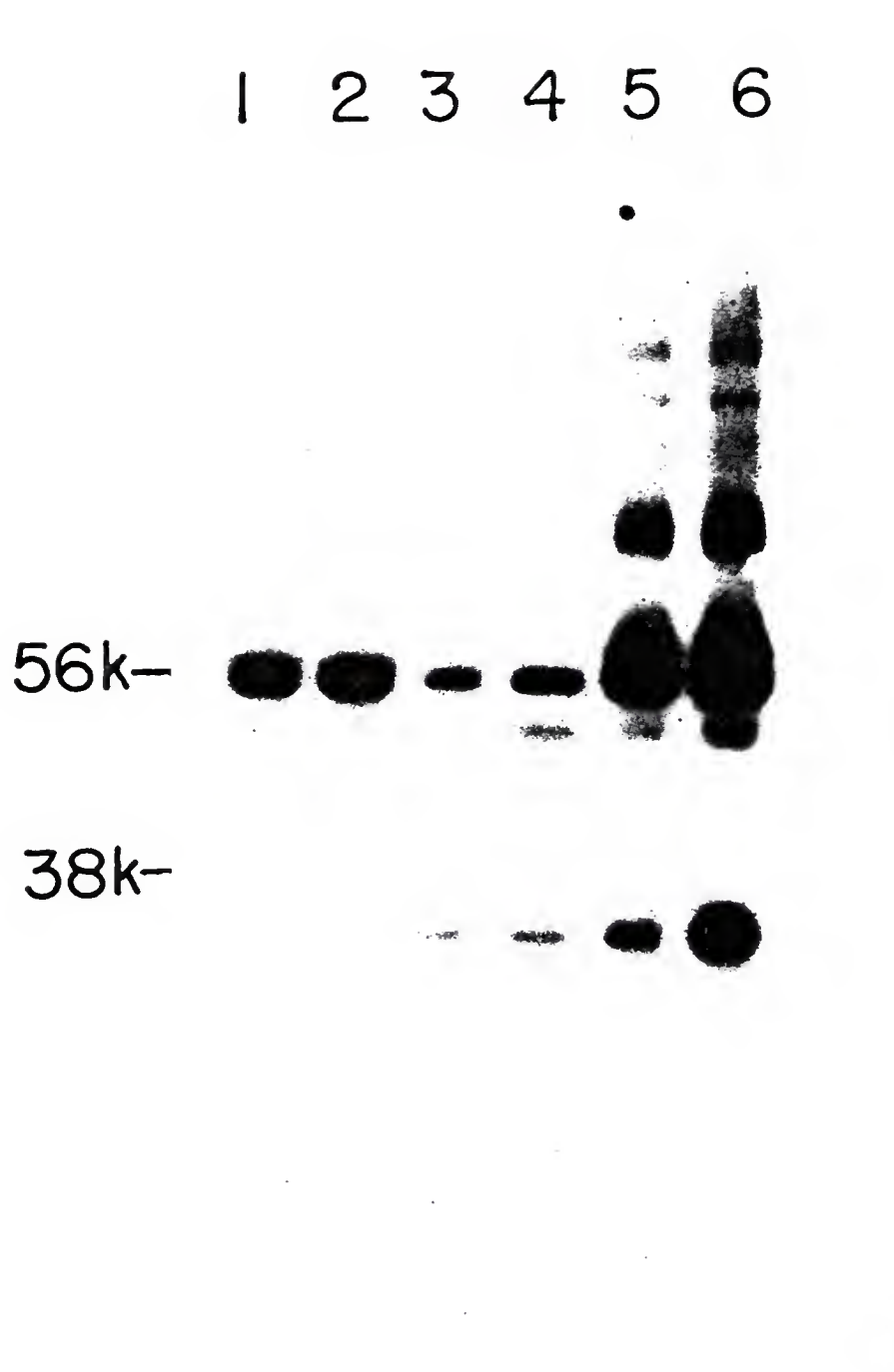


Fig. 3-1. Western blot autoradiograph of affinity purified extractions of 64/14/HRP.

Lanes 1 and 2 contain 1.8 µg and 4.2 µg of heat extracted Fc receptor. Lanes 3 and 4 contain 4 µg and 12 µg of hyaluronidase extracted Fc reactive material. Lanes 5 and 6 contain .5 µg and 1.2 µg mutanolysin extracted Fc receptor. The affinity purified samples were electrophoresed on an SDS polyacrylamide gel, electroblotted onto nitrocellulose, and probed with ^{125}I Fc specific probe as described in Materials and Methods. Autoradiography was for 20 hr at -70°C with an intensifying screen.

buffer. Other eluting buffers, including 1% SDS solution, 0.1 M glycine-HCl, pH 2.0 or 0.2 M glycyl tyrosine, were tested, but only $MgCl_2$ resulted in the recovery of significant quantities of functionally active Fc receptors.

After elution from immobilized IgG, each fraction was dialyzed overnight against PBS containing 10 mM EDTA to facilitate the removal of Mg^{++} and all samples were finally dialysed into PBS without EDTA. Each fraction was assayed for Fc receptor activity using a competitive binding assay. Fractions containing Fc receptor activity were pooled and concentrated by Amicon Ultrafiltration using a PM-10 membrane with a molecular weight cut off of 10,000 daltons. Aliquots were stored at -70°C and maintained their Fc receptor activity for at least 1 yr.

The size heterogeneity of the affinity purified Fc receptor was determined by electrophoresis on an SDS polyacrylamide gel followed by staining with Coomassie brilliant blue (Fig. 3-2A). Two protein bands were observed. The major protein band had a molecular weight of 56,000 daltons and a minor band was observed at 38,000 daltons. To determine if both bands had Fc receptor activity, a parallel SDS polyacrylamide gel was run, the separated proteins were electroblotted onto nitrocellulose and probed with the ^{125}I labeled Fc specific probe as described in Materials and Methods. The results of the Western blot demonstrated that the 56,000 dalton protein reacts strongly with the ^{125}I labeled Fc specific probe, whereas the 38,000 dalton protein demonstrated only weak reactivity (Fig. 3-2B).

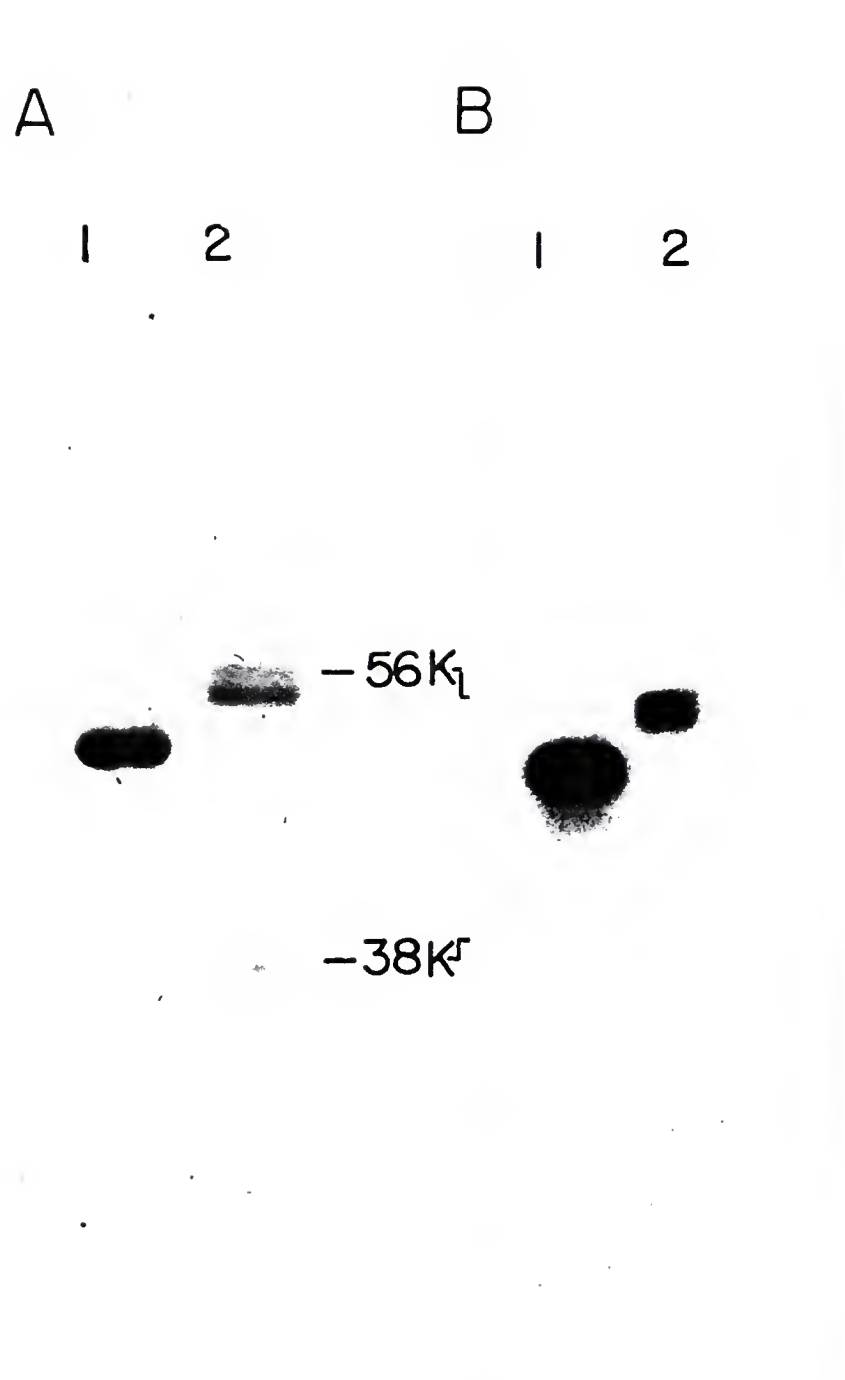


Fig. 3-2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the type II Fc receptor.

Panel A: Lane 1 contains 5 μg of the type I receptor (staphylococcal protein A). Lane 2 contains 4 μg of the type II Fc receptor. Samples were electrophoresed under denaturing conditions and stained with Coomassie brilliant blue as described in Materials and Methods. Panel B is the autoradiograph of a duplicate gel which was electroblotted onto nitrocellulose and probed with ^{125}I Fc specific probe. Autoradiography was for 2 days at -70°C using an intensifying screen. Lane 1 contains 30 ng of the type I receptor. Lane 2 contains 4000 ng of the type II receptor.

The Effect of Dipeptides on the Binding of IgG Subclasses to the Type II Fc Receptor

Certain amino acid residues in the Fc region of human IgG have been reported to be important in the binding of human IgG or certain human IgG subclasses to the type I Fc receptor (protein A). Tyrosine residues in the Fc region of human IgG (Deisenhofer et al., 1978) and in protein A (Sjoholm et al., 1973) have been shown to be involved in this interaction. The ability of protein A to bind certain allotypes of IgG_3 has been shown to be associated with a single amino acid in the heavy chain (Recht et al., 1981; Haake et al., 1982; Shimizu et al., 1983). Comparison of amino acid sequences of immunoglobulins that bind protein A with those that do not, has implicated the importance of histidine residue at position 435 of the heavy chain of the IgG_3 molecule (Haake et al., 1982). Furthermore, Bywater (1978,1983) reported that glycyl-tyrosine could elute human IgG bound to a protein A - sepharose column. When glycyl-tyrosine was tested for its ability to displace the bound type II Fc receptor from a human IgG affinity column, no Fc receptor activity was eluted. Glycyl-tyrosine and a second dipeptide, glycyl-histidine, however, did inhibit the binding of certain human IgG subclasses to the type II receptor-rich group A strain 64/14/HRP (Fig. 3-3). The binding of human subclasses 1, 2 and 4 to 64/14/HRP was inhibited by both glycyl-tyrosine and glycyl-histidine, whereas the effect of these two dipeptides on the binding of IgG_3 was minimal (Fig. 3-3). Glycylglycine had no effect on any of the subclasses and none of these dipeptides inhibited the uptake of labeled human IgG to the type I Fc receptor-positive Staphylococcus aureus Cowan strain, or to the type III Fc receptor-positive group C streptococcal strain 26RP66 (Fig. 3-4).

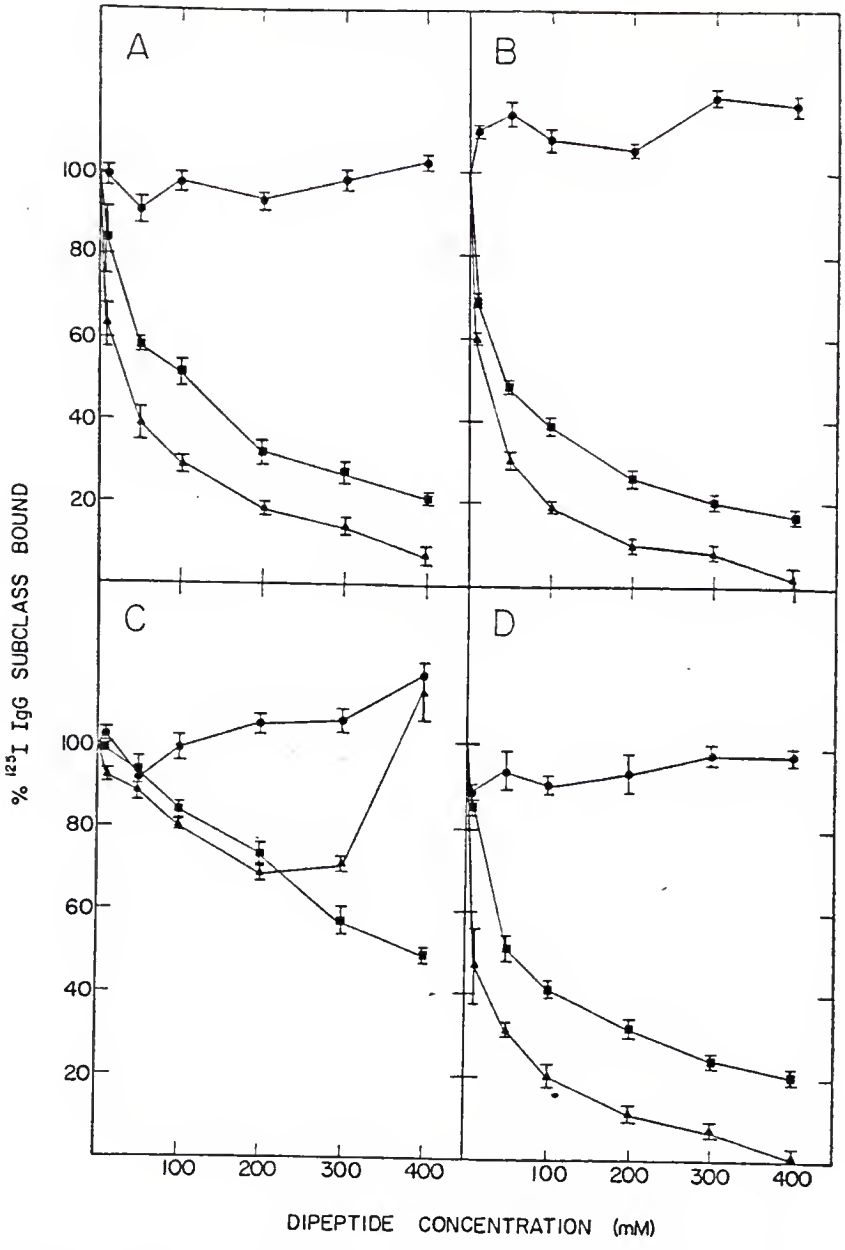


Fig. 3-3. The effect of dipeptides on the binding of ^{125}I-labeled human IgG subclasses to an Fc receptor-positive group A streptococcus.

A standard number of group A streptococci was incubated for 1.5 hours at 37°C with 20,000 cpm of the appropriately ^{125}I-labeled human IgG subclasses in the presence of varying concentrations of dipeptide. The bacteria were pelleted by centrifugation at 1000 x g for 10 min and washed twice with 3 ml of veronal buffered saline containing 0.01 M EDTA and 0.1% gelatin. The radioactivity associated with the bacteria was determined in an LKB autogamma counter. The cpm bound to the bacterial pellet were expressed as percent of maximum cpm bound.

Panel A represents the results obtained from the binding of ^{125}I IgG_1
Panel B represents the results obtained from the binding of ^{125}I IgG_2
Panel C represents the results obtained from the binding of ^{125}I IgG_3
Panel D represents the results obtained from the binding of ^{125}I IgG_4
●--● Glycylglycine
■--■ Glycyl-L-histidine
▲--▲ Glycyl-L-tyrosine

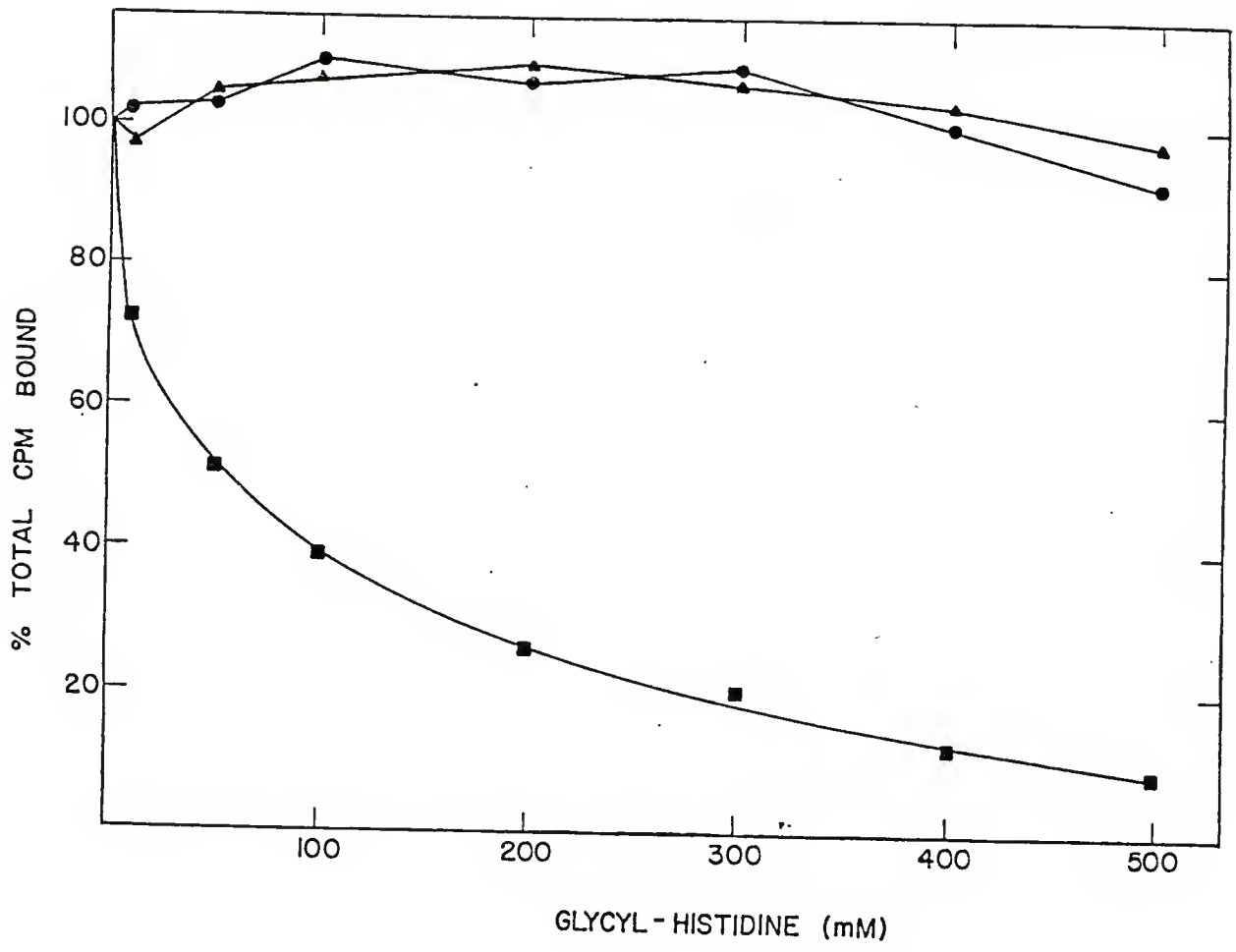


Fig. 3-4. The effect of glycyl-histidine on the binding of ^{125}I-labeled human IgG to three bacterial strains.

A standard number of Staphylococcus aureus Cowan I strain (▲-▲), group C streptococcal strain 26RP66 (●-●), or the group A streptococcal strain 64/14/HRP (■-■) was incubated for 1.5 hr at 37°C with 20,000 cpm of ^{125}I labeled human IgG in the presence of the indicated amounts of gycyl-histidine. The bacteria were pelleted by centrifugation at 1000 x g for 10 min and washed twice with 2 ml of veronal buffered saline containing 0.01 M EDTA and 0.1% gelatin. The radioactivity associated with the bacteria was determined in an LKB autogamma counter. The cpm bound to the bacterial pellet were expressed as percent of maximum cpm bound.

Reactivity of the Type II Fc Receptor With Human IgG Subclasses

The differences in the observed reactivity between the human IgG subclasses and the dipeptides (Fig. 3-3) raised the possibility that more than one Fc receptor existed on the surface of the group A strain 64/14/HRP. To test this possibility, samples containing the affinity purified heat extracted type II Fc receptor were electrophoresed on SDS polyacrylamide gels, transferred to nitrocellulose by electroblotting and parallel gels were probed with ^{125}I labeled human IgG_1, IgG_2, IgG_3, or IgG_4. The type I Fc receptor and the type III Fc receptor were included on each gel as reference positive controls (Fig. 3-5).

The type I Fc receptor, protein A, contained one major protein band with a M_r of approximately 52,000 that bound IgG_1, IgG_2, and IgG_4 but not IgG_3 (Fig. 3-5). The heat extracted, affinity purified type II receptor contained a major protein band with an M_r of approximately 56,000, and a minor band with a M_r of approximately 38,000. The major 56,000 dalton band reacted with human IgG_1, IgG_2, and IgG_4 but failed to react with human IgG_3. The minor 38,000 dalton protein reacted only with human IgG_3. The type III Fc receptor demonstrated a single protein band with a M_r of approximately 40,000 and this protein reacted with all four human IgG subclasses (Fig. 3-5).

A major concern with the results obtained in Figure 3-5, was the possibility that the observed reactivity of the type II receptor with IgG_3 was unique to the labeled myeloma probe being used. Consequently these studies were repeated using two other human IgG_3 myeloma proteins. The results shown in Figure 3-6 indicate that all

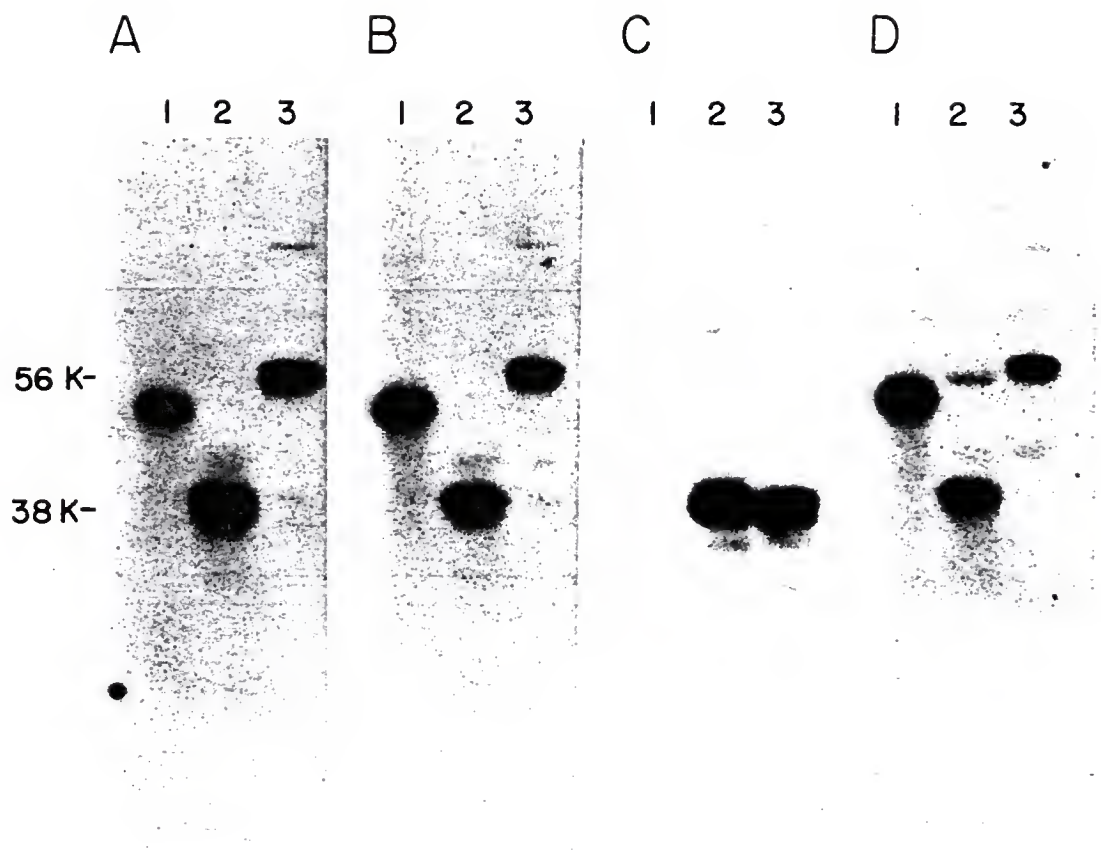


Fig. 3-5. Human immunoglobulin G subclass reactivity of bacterial Fc receptors.

Lane 1 contains 20 ng of the type I receptor (Staphylococcal Protein A). Lane 2 contains 100 ng of the type III Fc Receptor, and Lane 3 contains 4,000 ng of the type II Fc Receptor. Samples were electrophoresed under denaturing conditions in polyacrylamide gels containing sodium dodecyl sulfate according to Laemmli (13). Gels were equilibrated in 25mM tris, 192 mM glycine, pH 8.3 and 20% v/v methanol (transfer buffer) for 30 min. A piece of nitrocellulose previously soaked in the transfer buffer was placed on top of the gel. The gel and nitrocellulose were sandwiched between 2 pieces of Whatman 3mm paper and placed in a Bio-Rad trans blot appparatus with the nitrocellulose oriented between the anode and the gel. Electroblotting was at 70 volts for 3 hours in the above buffer. After electroblotting, the nitrocellulose was washed in veronal buffered saline containing 0.25% gelatin and 0.25% Tween-20 for 1 hour with four 250 ml changes. The nitrocellulose was probed for 3 hours in the washing buffer containing $2X10^5$ cpm/ml ^{125}I human IgG of the appropriate subclass. After probing the nitrocellulose was washed four times in 0.01M EDTA, 1M NaCl, 0.25% gelatin, and 0.25% Tween-20 for 15 min each and allowed to air dry. Autoradiography was carried out by exposure of the blot for 3 days at -70°C to Kodak XAR-5 film using an intensifying screen.

Panel A is the result from blots probed with ^{125}I IgG_1.
Panel B is the result from blots probed with ^{125}I IgG_2.
Panel C is the result from blots probed with ^{125}I IgG_3.
Panel D is the result from blots probed with ^{125}I IgG_4.

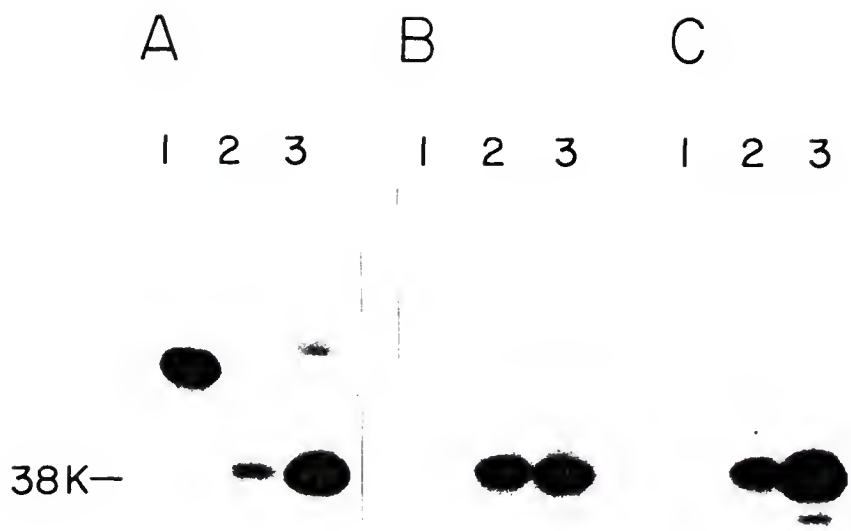


Fig. 3-6. Reactivity of three human myeloma IgG_3 samples with bacterial Fc receptors.

Lane 1 contains 20 ng of the type I receptor. Lane 2 contains 100 ng of the type III receptor and Lane 3 contains 4,000 ng of the type II receptor. Samples were electrophoresed on parallel SDS polyacrylamide gels, electroblotted onto nitrocellulose, and each blot was probed with a different ^{125}I labeled myeloma IgG_3 protein as decribed in the legend to Fig. 1. Autoradiography was carried out by exposure of the blot for 24 hours at -70°C to x-ray film using an intensifying screen.

Panel A represents a blot probed with ^{125}I IgG_3 (κ) Lot No. 0784.
Panel B represents a blot probed with ^{125}I IgG_3 (κ) Lot No. 0282.
Panel C represents a blot probed with ^{125}I IgG_3 (λ) Lot No. 0381.

three human IgG_3 samples reacted with the minor M_r 38,000 protein in the affinity purified heat extracts of the group A streptococcus. One of the IgG_3 samples was found to react with the type I receptor (staphylococcal protein A) and this particular sample of IgG_3 also reacted with the major M_r 56,000 protein as well as the minor M_r 38,000 protein in the extract of the group A streptococcus (Fig. 3-6, Panel A).

The IgG_3 reactivity was further confirmed in inhibition studies using unlabeled subclass standards. Only the sample containing IgG_3 could efficiently inhibit the binding of the ^{125}I labeled human IgG_3 to the type II receptor-positive group A streptococcus, while equimolar concentrations of IgG_1, IgG_2 or IgG_4 showed minimal inhibition (Fig. 3-7). All of the samples of unlabeled IgG_3 tested were capable of inhibiting the binding of the labeled IgG_3, with the most efficient inhibitor being the sample that was also used to prepare the labeled probe.

Separation of Two Functionally Distinct Type II Fc Receptors

The 56,000 dalton Fc receptor which reacted with IgG_1, IgG_2, and IgG_4 (designated type IIa), was separated from the 38,000 dalton IgG_3-specific Fc receptor (designated type IIb) by use of an immobilized column of IgG_3. Heat extracts of 64/14/HRP containing both Fc receptors were applied to a column of an isolated human IgG_3 (κ) myeloma immobilized on Affi-gel 15. The column was pre-equilibrated in 20 mM Tris-HCl, pH 7.5. The type IIa Fc receptor failed to bind to the IgG_3 column and was recovered in the void volume of the column by washing with the Tris-HCl buffer. The type IIb receptor, which bound to the $IgG3_3$ column, was eluted with 3 M

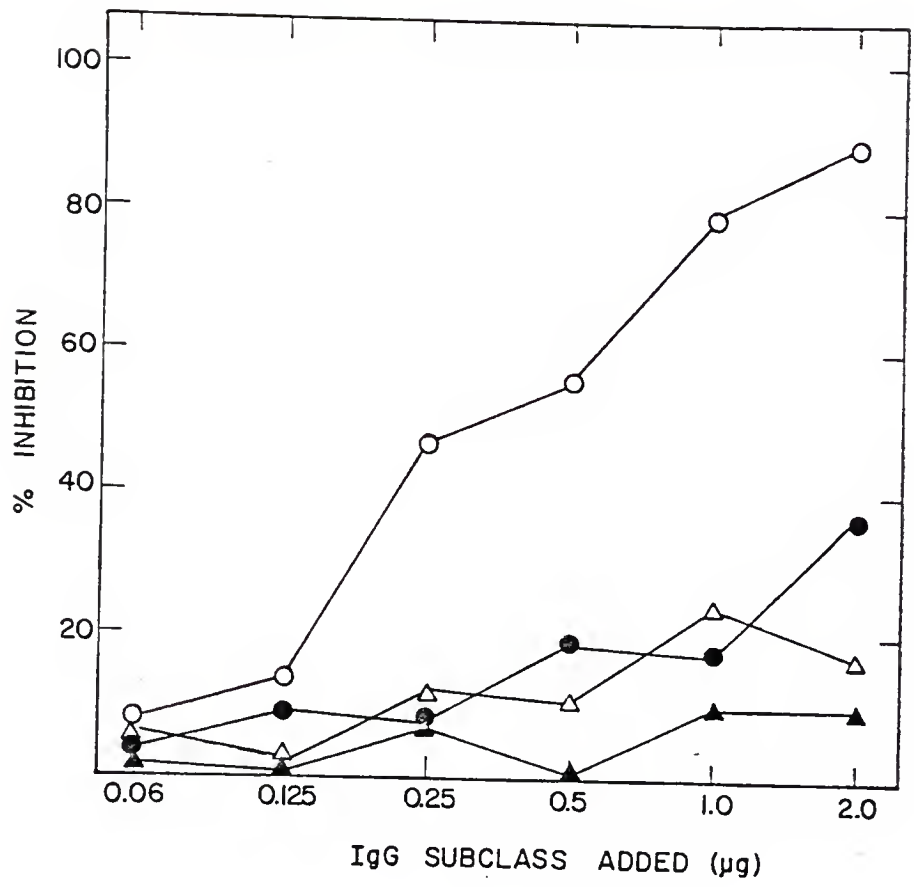


Fig. 3-7. Inhibition of binding ^{125}I labeled IgG_3 to a group A streptococcus by unlabeled human IgG subclasses.

Approximately 1 x 10^7 of the Fc receptor-rich group A streptococci, were incubated at 37°C for one hour with the indicated amounts of each human IgG subclass. Following incubation, each mixture was dotted onto a piece of nitrocellulose previously soaked in 25 mM tris, 192 mM glycine, pH 8.3 and 20% v/v methanol using the Bio-rad bio-dot microfiltration apparatus. After each well was washed with the above buffer, the nitrocellulose was removed and washed in veronal buffered saline containing 0.25% gelatin and 0.25% Tween-20 (wash buffer) for one hour with four 250 ml changes. The nitrocellulose was probed for three hours in the wash buffer containing 2 x 10^5 cpm/ml ^{125}I labeled human IgG_3. After probing the nitrocellulose was washed four times in 0.01M EDTA, 1M Na Cl, 0.25% gelatin and 0.25% Tween-20 and allowed to air dry. The nitrocellulose was cut into sections that contained an individual well and the cpm associated with each section of nitrocellulose was determined using an LKB autogamma counter and the percent inhibition of ^{125}I IgG_3 binding to the bacteria was calculated.

●--● IgG1
△--△ IgG2
○--○ IgG3
▲--▲ IgG4

$MgCl_2$ in 20 mM Tris-HCl, pH 7.5, dialyzed, and concentrated as described above.

The unbound material from the IgG_3 column (the type IIa receptor) was further purified by binding to, and eluting from a column of immobilized human IgG. The affinity purified Fc receptors were analyzed by the Western blotting procedure. The type IIa and type IIb Fc receptors were electrophoresed on SDS polyacrylamide gels, electroblotted onto nitrocellulose and parallel gels were probed with either ^{125}I labeled human IgG to detect the type IIa Fc receptor, or ^{125}I labeled human IgG_3 to detect the type IIb Fc receptor (Fig 3-8). ^{125}I labeled human IgG was used as a probe for the type IIa Fc receptor because of the distribution of subclasses 1, 2, and 4 in relation to subclass 3 found in normal human serum. Human IgG subclass 3 comprises only 8% of the total human IgG in the serum (Lewis et al., 1970). Therefore, the contribution made by IgG subclass 3 in the labeled human IgG probe is minimal.

In Fig. 3-8A, the type IIa Fc receptor reacted strongly with the human IgG probe and no contamination with the type IIb Fc receptor was seen on the gel probed with ^{125}I labeled IgG_3 (Fig. 3-8B). Similarly, the isolated type IIb Fc receptor reacted strongly with the IgG_3 probe (Fig. 3-8B) and no contamination of the type IIa Fc receptor was seen (Fig. 3-8A). The type I and type III Fc receptors were included on each gel as reference positive controls.

Species Immunoglobulin Reactivity With the Type IIa and Type IIb Fc Receptors

Five distinct bacterial Fc receptors have been classified based on the reactivity of whole bacteria with different sources of

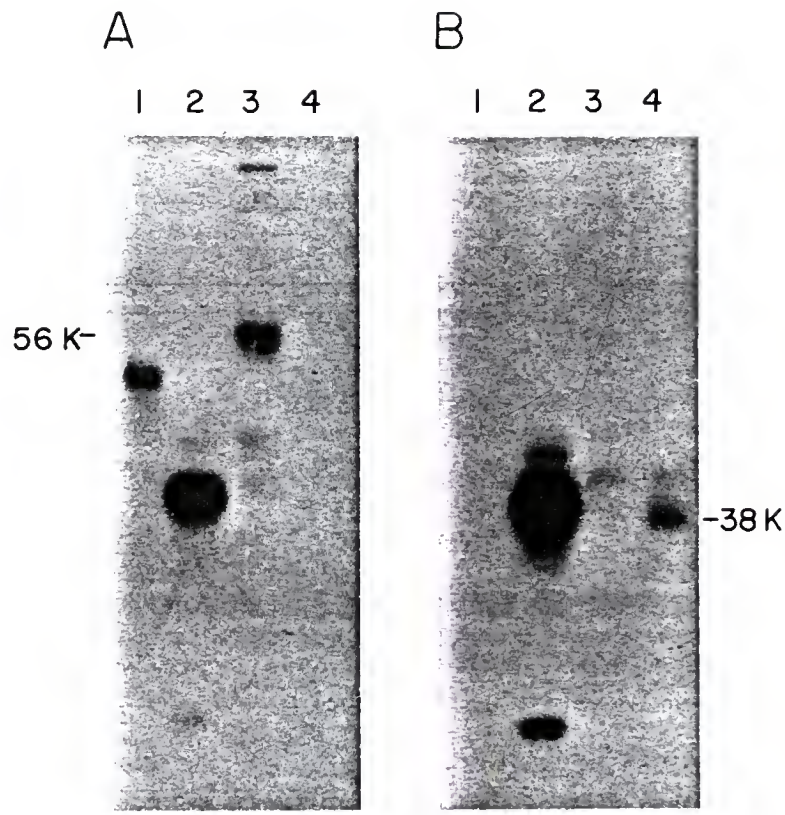


Fig. 3-8. Separation of the type IIa Fc receptor from the type IIb Fc receptor.

Lane 1 contains 30 ng of the type I Fc receptor (protein A). Lane 2 contains 30 ng of the type III Fc receptor. Lane 3 contains 3 µg of the type IIa Fc receptor, and Lane 4 contains 2 µg of the type IIb Fc receptor. Samples were electrophoresed on SDS polyacrylamide gels, electroblotted onto nitrocellulose, and probed with either ^{125}I labeled human IgG or IgG_3. Panel A represents a blot probed with ^{125}I human IgG. Panel B represents a blot probed with ^{125} human IgG_3.

immunoglobulins (Myhre and Kronvall, 1981). In this classification, the type II Fc receptor associated with group A streptococci should bind all four subclasses of human IgG, and IgG from rabbit and pig (Myhre and Kronvall, 1977) (see Table 1-1). In the next series of experiments, the IgG species binding profile for the group A strain 64/14/HRP which was used to isolate the type IIa and type IIb Fc receptors was determined. A standard number of group A strain 64/14/HRP was incubated with increasing concentrations of dog, goat, sheep, rat, pig, rabbit, bovine, or human IgG. After incubation, the mixture was dotted onto nitrocellulose and the nitrocellulose was then probed with either ^{125}I labeled human IgG, or ^{125}I labeled human IgG_3. Details are described in Materials and Methods. The results indicate that the Fc receptors on the surface of group A strain 64/14/HRP could only bind pig, rabbit, and human IgG. Dog, goat, sheep, rat or bovine IgG could not inhibit the binding of ^{125}I labeled human IgG (Fig. 3-9A) or ^{125}I labeled human IgG_3 (Fig. 3-9B) to strain 64/14/HRP.

The ability of pig and rabbit IgG to inhibit the binding of both ^{125}I labeled human IgG and ^{125}I labeled human IgG_3 indicates that either the type IIa and type IIb Fc receptors on the surface of group A strain 64/14/HRP are both capable of reacting, or both Fc receptors are linked on the bacterial surface and the binding of human, pig, or rabbit IgG to one Fc receptor sterically hinders the ability of the other Fc receptor to bind.

The purpose of the next experiment was to determine if both the type IIa and type IIb Fc receptors reacted equally with rabbit and pig IgG, or if the inhibition seen in Fig. 3-9 with both ^{125}I labeled human IgG and human IgG_3 was due to steric hindrance.

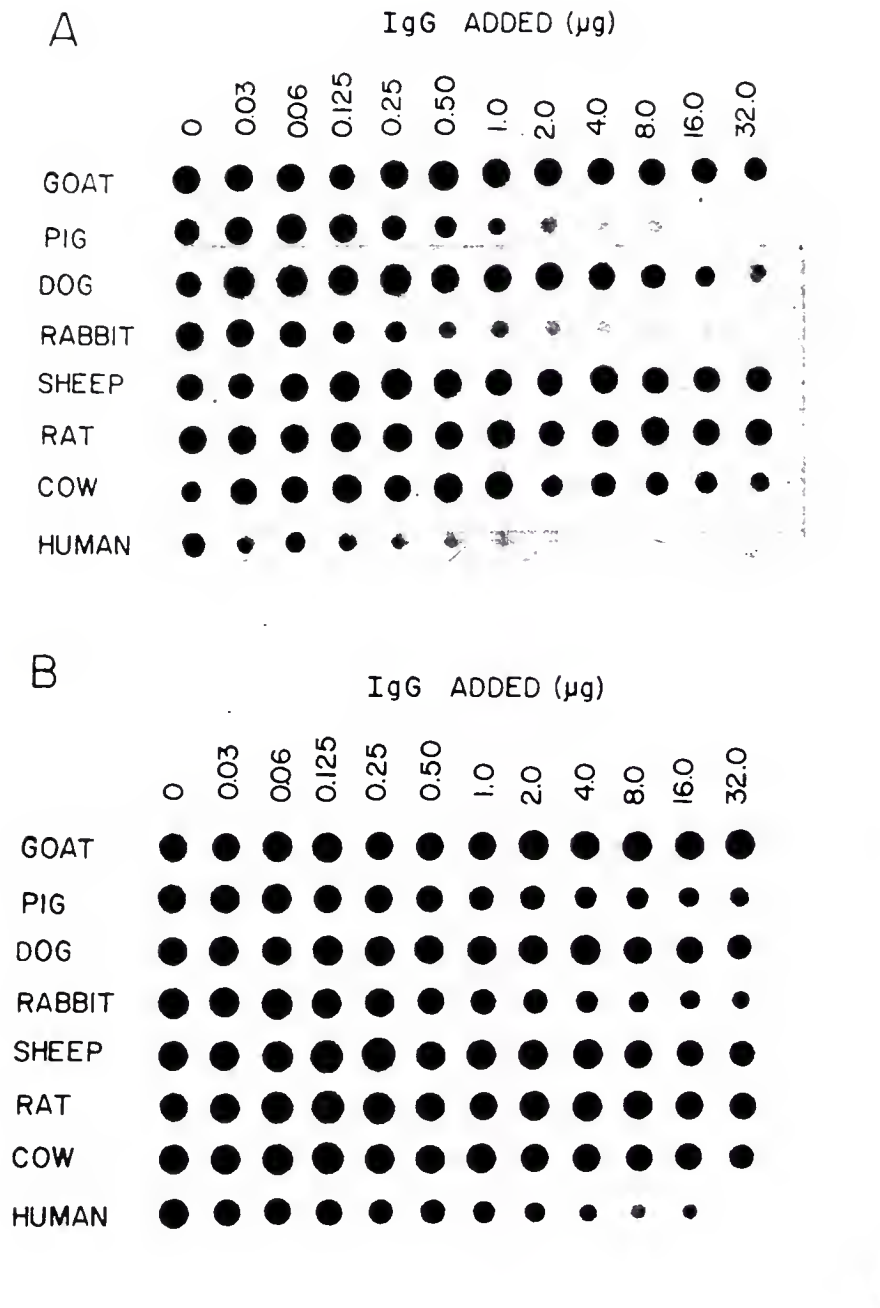


Fig. 3-9. Inhibition of ^{125}I human IgG or ^{125}I human IgG_3 to group A strain 64/14/HRP by immunoglobulin G from a variety of mammalian species.

A standard number of group A strain 64/14/HRP was incubated at 37°C for 1 hr with the indicated amount of goat, pig, dog, rabbit, sheep, rat, cow, or human IgG. Following incubation, each mixture was dotted onto nitrocellulose. The nitrocellulose was washed and probed with ^{125}I labeled human IgG or human IgG_3 as described in Materials and Methods. Autoradiography was for 16 hr at -70°C with an intensifying screen.

Panel A represents the blot probed with ^{125}I labeled human IgG.
Panel B represents the blot probed with ^{125}I labeled human IgG_3.

Samples containing both the type IIa and type IIb Fc receptors were electrophoresed on SDS polyacryladmide gels, electroblotted onto nitrocellulose and probed with ^{125}I labeled human IgG, pig IgG, rabbit IgG, dog IgG, or bovine IgG (Fig. 3-10). The results show that only the type IIa Fc receptor could bind ^{125}I labeled pig or rabbit IgG. Reactivity with the type IIb Fc receptor was only seen with ^{125}I labeled human IgG (Fig. 3-10). This suggests that the two Fc receptors must be closely linked on the surface of group A strain 64/14/HRP to account for the partial inhibition of ^{125}I labeled IgG_3 that is seen in Fig. 3-9B.

Antigenic Relationship Between the Type IIa and Type IIb Fc Receptors

In order to test whether both the type IIa and type IIb Fc receptors were antigenically related, antibodies were prepared in chickens against the type IIa and type IIb Fc receptors. The affinity purified heat extract, containing both Fc receptors, was electrophoresed on an SDS polyacrylamide gel and both the 56,000 dalton Fc receptor and 38,000 dalton Fc receptor were identified by Coomassie blue staining. Each band was cut from the gel and used as the immunogen to prepare the monospecific antibodies using the immunization schedule described in Materials and Methods. Antibody was isolated from the egg yolk by chloroform extraction and activity was monitored by the ability of the isolated extract to inhibit binding of ^{125}I human IgG or human IgG_3 to the group A strain 64/14/HRP. The results in Fig. 3-11A indicate that the antibody prepared against the type IIa (M_r = 56,000) Fc receptor can efficiently inhibit the binding of both ^{125}I labeled human IgG and ^{125}I labeled human IgG_3 to the group A strain 64/14/HRP. A similar result was observed using the

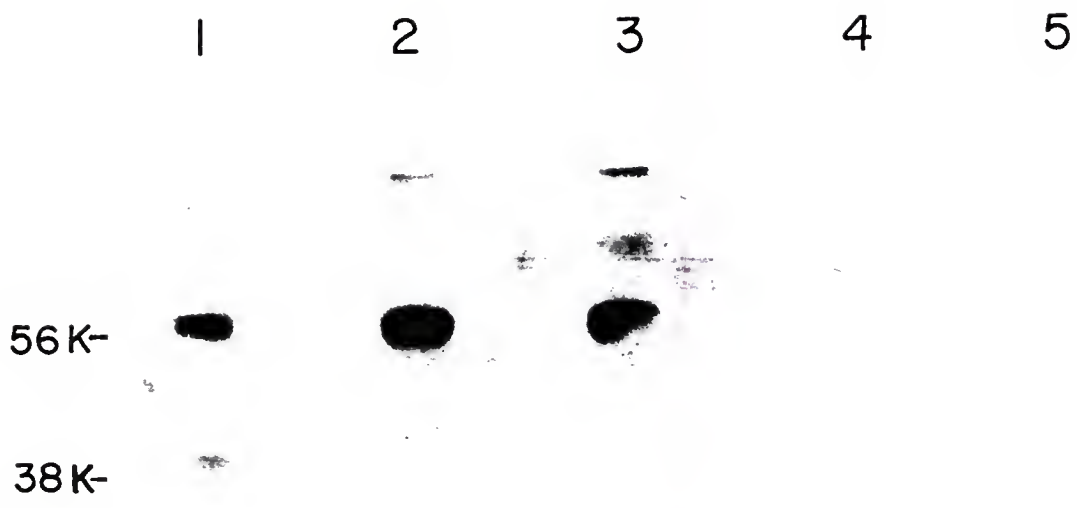


Fig. 3-10. Reactivity of IgG from a variety of species with the type IIa or type IIb Fc receptor.

Affinity purified heat extract (4 μg) containing both the type IIa and type IIb Fc receptors was electrophoresed on five parallel SDS polyacrylamide gels, electroblotted onto nitrocellulose and probed with ^{125}I labeled human IgG (Lane 1), pig IgG (Lane 2), rabbit IgG (Lane 3), dog IgG (Lane 4), or bovine IgG (Lane 5) as described in Materials and Methods. Autoradiography was for 30 hr at -70°C using an intensifying screen.

anti-type IIb Fc receptor (Fig. 3-11B). No inhibition was observed when normal chicken immunoglobulins were added. The shapes of the inhibition curves of the IgG binding or the IgG_3 binding were similar for each antibody (compare Figures 3-11A and 3-11B). This finding suggests that either each Fc receptor was present at an approximately equal density and recognized with equivalent affinity, or that the two Fc receptors were closely linked on the bacterial surface. When the heat extract was affinity purified by binding to a column of immobilized chicken anti-type IIb Fc receptors and then eluting with 3 M $MgCl_2$, both the type IIa and type IIb Fc receptors were recovered in a functionally active form (data not shown). This provides evidence that the two distinct Fc receptors are antigenically related and that the observed inhibition by the anti-type IIa and type IIb antibodies on the intact group A organism could not solely be attributed to steric hindrance.

Antigenic Relationship of the Type I, Type II and Type III Fc Receptors

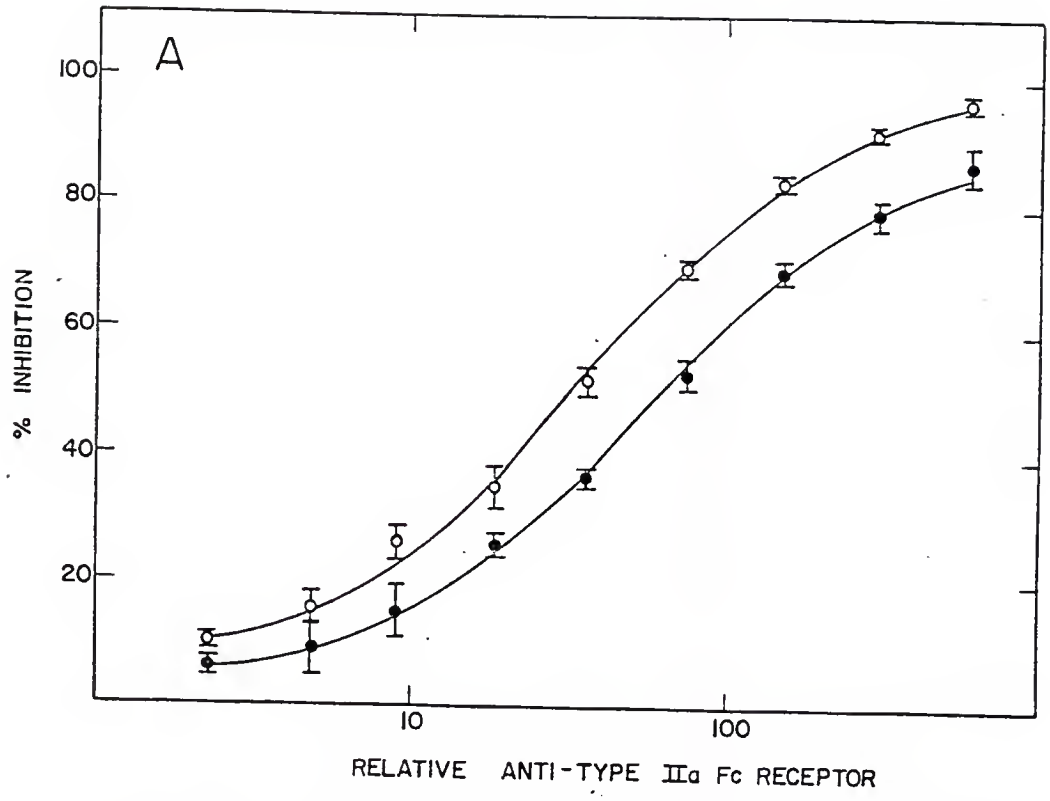
The antigenic relationship between the type I, II and III bacterial Fc receptors was tested using monospecific chicken antibodies to each receptor type. Each antibody was tested at a number of concentrations for its ability to inhibit the binding of ^{125}I labeled human IgG to a fixed concentration of the group A streptococcal strain 64/14/HRP. The results, presented in Fig. 3-12, indicate that only the antibody against the type II Fc receptor could inhibit the binding of ^{125}I labeled human IgG to strain 64/14/HRP. Antibodies against the type I Fc receptor could not inhibit the binding of ^{125}I labeled human IgG to the group A strain 64/14/HRP or to the

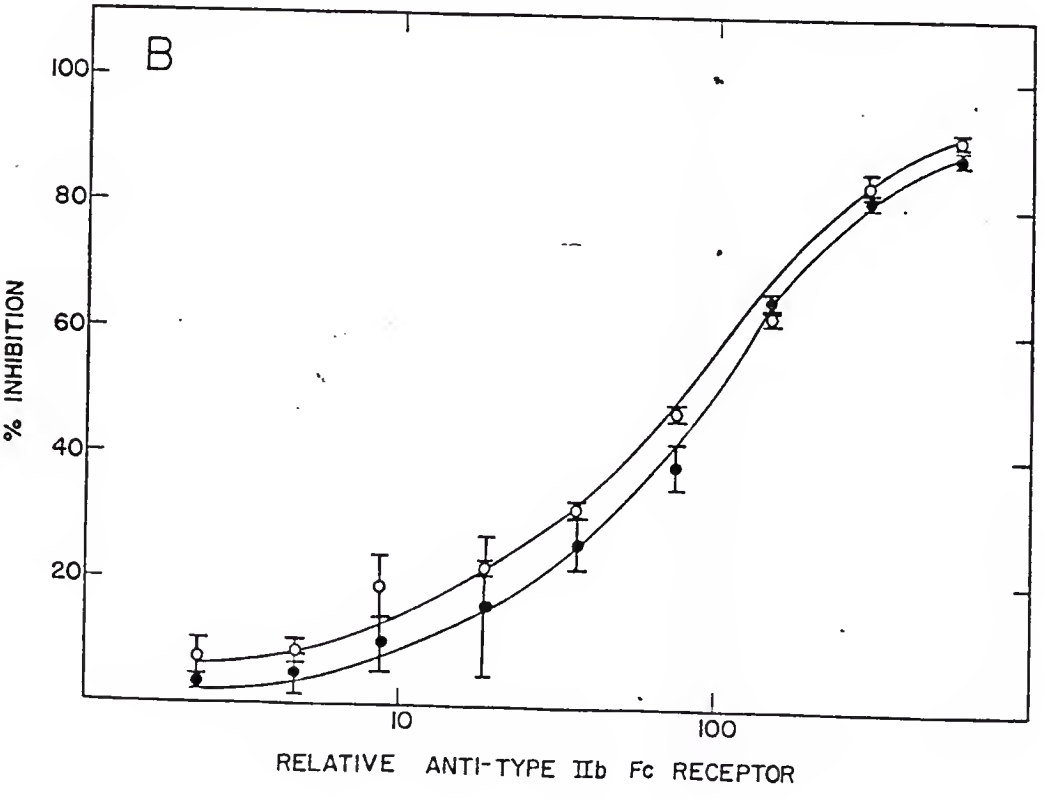
Fig. 3-11. Inhibition of binding of ^{125}I-labeled human IgG or ^{125}I-labeled human IgG subclass 3 to group A streptococcal strain 64/14/HRP by monospecific antibodies against the purified type IIa Fc receptor or the type IIb Fc receptor.

A standard number of group A streptococcal strain 64/14/HRP was incubated 1.5 hr at 37°C with 20,000 cpm of ^{125}I labeled human IgG or ^{125}I labeled human IgG_3 in the presence of the indicated amounts of the monospecific chicken anti-type IIa (Panel A) or anti-type IIb Fc receptor (Panel B). The bacteria were pelleted by centrifugation at 1000 x g for 10 min and washed twice with 2 ml veronal buffered saline containing 0.01 M EDTA and 0.1% gelatin. The radioactivity associated with the bacteria was determined in an LKB autogamma counter and the percent inhibition calculated.

o--o ^{125}I labeled human IgG_3

●--● ^{125}I labeled human IgG


A
100
80
60
40
20
% INHIBITION
10
100
RELATIVE ANTI-TYPE IIa Fc RECEPTOR


B
100
80
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% INHIBITION
10
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RELATIVE ANTI-TYPE IIb Fc RECEPTOR

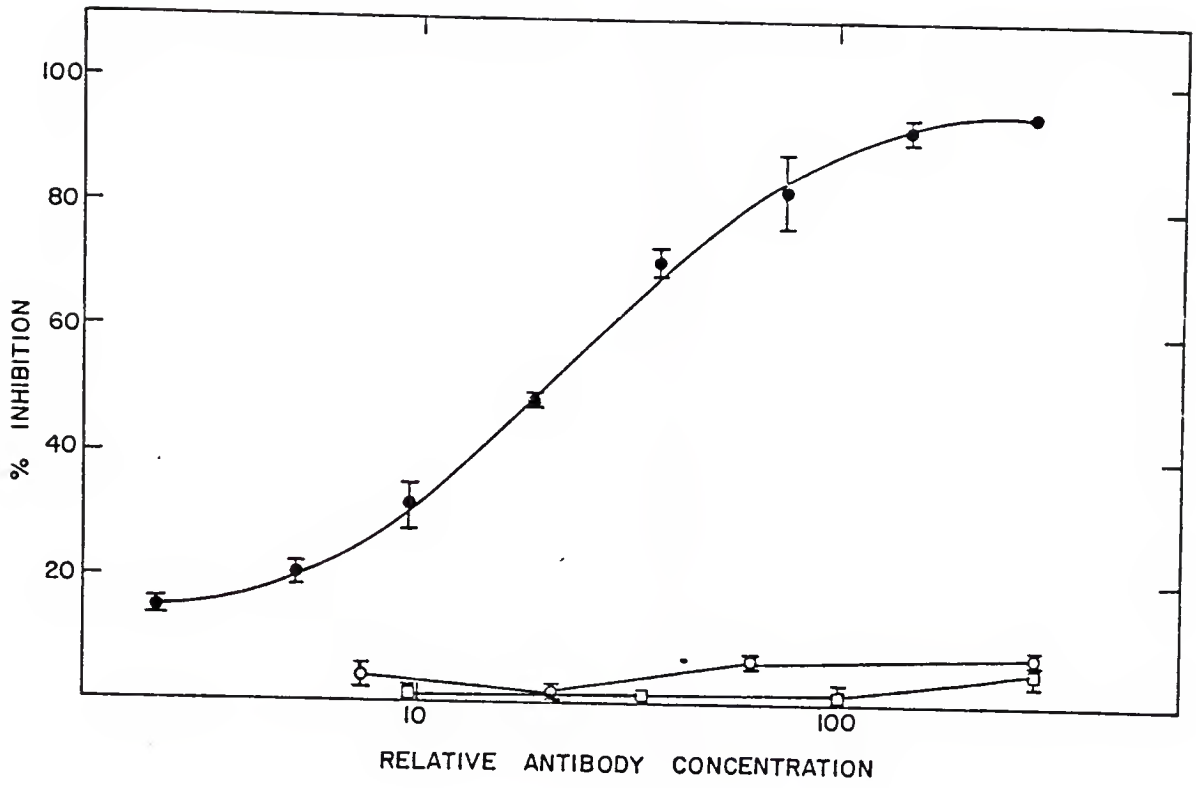


Fig. 3-12. Inhibition of binding of ^{125}I human IgG to 64/14/HRP by antibody against the type I receptor (□-□), the type II receptor (●-●), or the type III receptor (o-o).

A relative antibody concentration of 100 for the type I antibodies inhibits the binding of ^{125}I human IgG to the Staphylococcus aureus Cowan I strain by 94% and inhibits the binding of ^{125}I human IgG to the group C streptococcal 26RP66 strain by less than 10%.

A relative antibody concentration of 100 for the type III antibody inhibits the binding of ^{125}I human IgG to the Staphylococcus aureus Cowan Strain by less than 10% and inhibits the binding of ^{125}I human IgG to the group C streptococcal 26RP66 strain by 92%.

group C Fc receptor-rich strain 26PP66, but could completely inhibit the binding of the labeled immunoglobulin to the Staphylococcus aureus Cowan strain. Similarly, the antibody to the type III receptor failed to inhibit binding of ^{125}I human IgG to either the group A streptococcal strain 64/14/HRP or to the Staphylococcus aureus Cowan strain while totally inhibiting the binding of labeled immunoglobulin to the Fc receptor-rich group C streptococcus 26RP66. No inhibition of binding was observed to any bacterial strain when normal chicken immunoglobulins were added.

Discussion

In this Chapter I describe the isolation and characterization of two functionally active Fc receptors from an Fc receptor-rich substrain of a group A streptococcus. This bacterial substrain has been selected from a mouse-passaged group A strain by use of an immunoblotting technique that measures Fc receptor expression on individual colonies (Chapter Two). Several methods for solubilizing the Fc receptor from this bacteria were tested, including: 1) heat extraction at neutral, acid or alkaline pH, 2) digestion with the enzymes mutanolysin, hyaluronidase, papain, trypsin, or phage lysin, or 3) autoclaving or heating the bacteria in the presence of SDS. Soluble Fc receptor activity was detected after heat extraction at neutral pH, treatment with mutanolysin or hyaluronidase, or following autoclaving or heating in the presence of SDS, Table 3-1. No activity was recovered following any of the other extraction procedures (see Table 3-1). Heat extraction at neutral pH resulted in the most homogeneous form of the receptor, (see Fig. 3-1) and this also had one of the highest yields of any of the extraction procedures tested (see Table 3-1).

Functionally active Fc receptor activity could be isolated from the heat extract by binding to and elution from a column of immobilized human IgG. A variety of different agents were tested for their ability to remove the bound receptor from the immobilized human IgG column and the most efficient was found to be 3 M $MgCl_2$. Analysis of the affinity purified material by SDS polyacrylamide gel electrophoresis revealed two bands with molecular weights of 56,000 and 38,000 daltons (Fig. 3-2). When the proteins were electroblotted onto nitrocellulose and probed with a ^{125}I human IgG Fc specific probe, both bands were shown to be active, although the 38,000 dalton band had much less activity. These two proteins bands could be shown to be antigenically related, since both were recovered when the heat extract was affinity purified on a column of immobilized chicken antibody that was directed against a single form of the affinity purified Fc receptor. Although these findings would be consistent with the 38,000 dalton protein being a degradation product of the larger 56,000 dalton receptor, protease inhibitors were included in all buffers used during the purification.

Another possibility was that these two molecular weight forms were distinct Fc receptors. Recent studies by Wagner et al. (1983) using immunoelectronmicroscopic approaches suggest that several distinct Fc receptors can be present on the surface of a single group A streptococcus. Analysis of the effects of various dipeptides (Fig. 3-3) and the reactivity of different human IgG subclasses (Fig. 3-5) confirmed the existence of at least two distinct Fc receptors on the group A strain being studied.

The identification of two distinct Fc receptors from group A strain 64/14/HRP was shown by probing four parallel nitrocellulose blots containing the affinity purified type II Fc receptors with each human IgG subclass. The 56,000 dalton protein (designated as type IIa Fc receptor) could bind human IgG subclasses 1, 2, and 4, whereas the 38,000 dalton protein (designated as type IIb Fc receptor) only bound human IgG subclass 3 (Fig. 3-5). Two other IgG_3 myeloma proteins were also tested and both reacted with the 38,000 dalton receptor (Fig. 3-5). Only one of the IgG_3 myeloma proteins tested was capable of reacting with the type I Fc receptor and this immunoglobulin also demonstrated a low level of reactivity with the 56,000 dalton (type IIa) receptor (Fig. 3-6A). This suggests that the amino acid residues which are important in the binding of the Fc region of human IgG_1, IgG_2, IgG_4, and certain allotypes of IgG_3, to the type I Fc receptor, may also be part of the recognition site on the IgG molecule for the type IIa Fc receptor. In addition, the type IIa Fc receptor was capable of binding pig and rabbit IgG in contrast to the type IIb Fc receptor which could only bind human IgG_3 (Fig. 3-10).

The studies on the effect of dipeptides on the binding of certain human IgG subclasses to the Fc receptors on the surface of group A strain 64/14/HRP provided further evidence for two distinct functionally active forms of Fc receptor. Significant inhibition of binding of IgG_1, IgG_2 and IgG_4 to 64/14/HRP was observed with glycyl-histidine and glycyl-tyrosine, while IgG_3 binding was not markedly effected (Fig. 3-3). Tyrosine and histidine are known to be important residues in the binding of IgG to the type I Fc receptor

(Deisenshofer et al., 1978; Recht et al., 1981; Haake et al., 1982; Shimizu et al., 1983), but in these studies only the type IIa receptor was inhibited. No effects were observed on the interaction of IgG with either the type I or type III Fc receptors (Fig. 3-4).

Although the type IIa and type IIb Fc receptors are functionally and physicochemically distinct, they were shown to be antigenically related (Fig. 3-11). Monospecific antibodies prepared against the type IIa or type IIb Fc receptors showed similar patterns of inhibition of the binding of both ^{125}I labeled human IgG and ^{125}I labeled human IgG_3 to the group A strain 64/14/HRP. Also, both Fc receptors were recovered when the heat extract was affinity purified on a column of immobilized anti-type IIb Fc receptor, indicating that the similar inhibition curves produced with either monospecific antibody could not solely be attributed to steric hindrance of closely linked Fc receptors on the bacterial surface. Based on the results of the inhibition study using pig and rabbit IgG (Fig. 3-9), however, the type IIa and type IIb Fc receptors are probably located close to each other on the cell surface.

To date, there have been two other reports describing the isolation of an Fc receptor from group A streptococci. Havlicek (1978) described the isolation of an Fc receptor from Streptococcus pyogenes which had a molecular weight of approximately 100,000 daltons and was recovered from the bacteria following acid extraction. Grubb et al. (1982) reported the isolation of a type II receptor from a group A streptococci, type 15, following alkaline extraction. This receptor could only be isolated to homogeneity in the presence of high concentrations of protease inhibitors and had an apparent molecular

weight of 29,500. The results presented in Table 3-1 indicate that I was unable to recover Fc receptor activity following either acid or alkaline extraction of the group A strain used. These findings would suggest that the Fc receptors on group A streptococci may represent a heterogeneous group of molecules which are antigenically related.

The group A streptococcal Fc receptors I have described are antigenically and physicochemically distinct from Fc receptors isolated from other bacteria. Antibodies prepared against the type I staphylococcal or type III group C streptococcal Fc receptors failed to react with the group A Fc receptor and all three receptors had different molecular weights, see Figures 3-5 and 3-12.

The studies reported here suggest that at least two functionally and physicochemically distinct Fc receptors are present on the surface of certain group A streptococci. To date, using an immunoblotting technique to study expression of Fc receptors on individual bacterial colonies (Chapter Two) I have been unable to find a strain that expressed only the IgG_3 selective Fc receptor. The abilty to isolate such a subclass selective receptor has a variety of important practical applications for the isolation and quantitation of human IgG_3. The importance of such receptors in the pathogenesis of certain streptococcal infections is unclear. The interaction between bacterial products and components of the host immune system may explain some of the post infection sequelae associated with infection by certain group A streptococci.

CHAPTER FOUR
DISTRIBUTION OF THE TYPE II Fc RECEPTORS ON NEPHRITOGENIC AND NON-NEPHRITOGENIC GROUP A STREPTOCOCCI

Introduction

The importance of Fc receptors in the course of bacterial infections and post-infection sequelae is not clear. Bacterial Fc receptors have been postulated as virulence factors (Ginsberg, 1972; Schalen, 1982; Christensen et al., 1977,1978,1981) and a correlation has been reported between the virulence of certain group A streptococci and their ability to bind the Fc portion of human IgG (Burova et al., 1980). Post-streptococcal glomerulonephritis, a complication that occasionally occurs following a group A streptococcal infection, is believed to result from deposition of complement fixing immune complexes in the glomeruli which initiate a complex series of reactions that result in the development of renal lesions (Levinsky, 1981).

In the studies described in this chapter, a series of nephritogenic and non-nephritogenic group A streptococci were screened for Fc receptor expression. This study was designed to determine whether Fc receptors are more frequently associated with group A strains with nephritogenic M serotypes, than with those of M serotype not associated with nephritogenic potential.

In Chapter Two, I have described a sensitive blotting technique that detects Fc receptor expression on bacterial surfaces. In this chapter I have applied a modification of this technique to study the

interaction of human IgG or human IgG subclasses with group A streptococci recovered from patients who either did, or did not develop post-streptococcal glomerulonephritis.

Materials and Methods

Streptococcal Strains

Eighteen of the streptococcal strains used in this study were obtained from the Rockefeller University collection and were a gift from Dr. Vincent Fischetti. Fifteen of these strains were isolated from patients with post-streptococcal glomerulonephritis. The characteristics of each of these strains have been reported in detail by Villarreal et al. (1979). Other group A streptococal strains were obtained from Dr. Elia Ayoub at the University of Florida, College of Medicine and were either throat or skin isolates.

Dot-Blotting Procedure

Dot blots were performed using the Bio-Rad bio-dot microfiltration apparatus and a modification of the Bio-Rad procedure. A piece of nitrocellulose previously soaked in 25 mM tris, 192 mM glycine, pH 8.3 and 20% v/v methanol (wash buffer) was placed in the apparatus. Two-fold serial dilutions of the bacteria were pipetted into the wells. The bacteria were diluted in the wash buffer, starting with approximately 1 x 10^8 bacteria. The concentration of organisms was standardized by measuring the optical density at 550 nm. After washing the bacteria in each well with the above buffer, the nitrocellulose was removed and washed four times in veronal buffered saline (VBS), pH 7.35, containing 0.25% gelatin and 0.25% Tween-20. Each wash was carried out for a period of 15 minutes using 250 ml of buffer. The

nitrocellulose was then probed for three hours in the washing buffer containing 2 x 10^5 cpm/ml of the appropriate ^{125}I labeled human IgG or human IgG subclass. After probing, the nitrocellulose was washed four times in 0.01 M EDTA, 1 M NaCl, 0.25% gelatin, and 0.25% Tween-20 (15 minutes each wash) and allowed to air dry. All washing and probing steps were performed at ambient temperature. The nitrocellulose blots were autoradiographed by exposing to Kodak XAR-5 film with an intensifying screen for 3-5 days at -70°C.

Iodination

Human IgG and human IgG subclases were iodinated as described in Chapter Three.

Results

The distribution of Fc receptors on the surface of 35 strains of group A streptococci was studied using a dot-blotting procedure. The results of the experiment probing nephritogenic and non-nephritogenic group A strains with ^{125}I labeled human IgG are presented in Fig. 4-1 and the corresponding radioactive counts for the individual sample wells are presented in Table 4-1. A comparison of the results in Fig. 4-1 with the counts in Table 4-1 indicate that the intensity of the spot on the autoradiograph correlates closely with the number of ^{125}I counts bound to the bacteria attached to the nitrocellulose. Binding was shown to be dependent on the concentration of bacteria used in the assay, and non-specific binding was found, under the experimental conditions chosen, not to be significant (Table 4-1). A similar relationship between the intensity of the spot on an autoradiograph and the counts bound to the bacteria was observed with all of the labeled probes tested.

Fig. 4-1. Binding of ^{125}I human IgG to nephritogenic and non-nephritogenic group A streptococci.

The indicated numbers of bacteria were dotted onto nitrocellulose and probed with ^{125}I human IgG as described in Materials and Methods.

Panel A

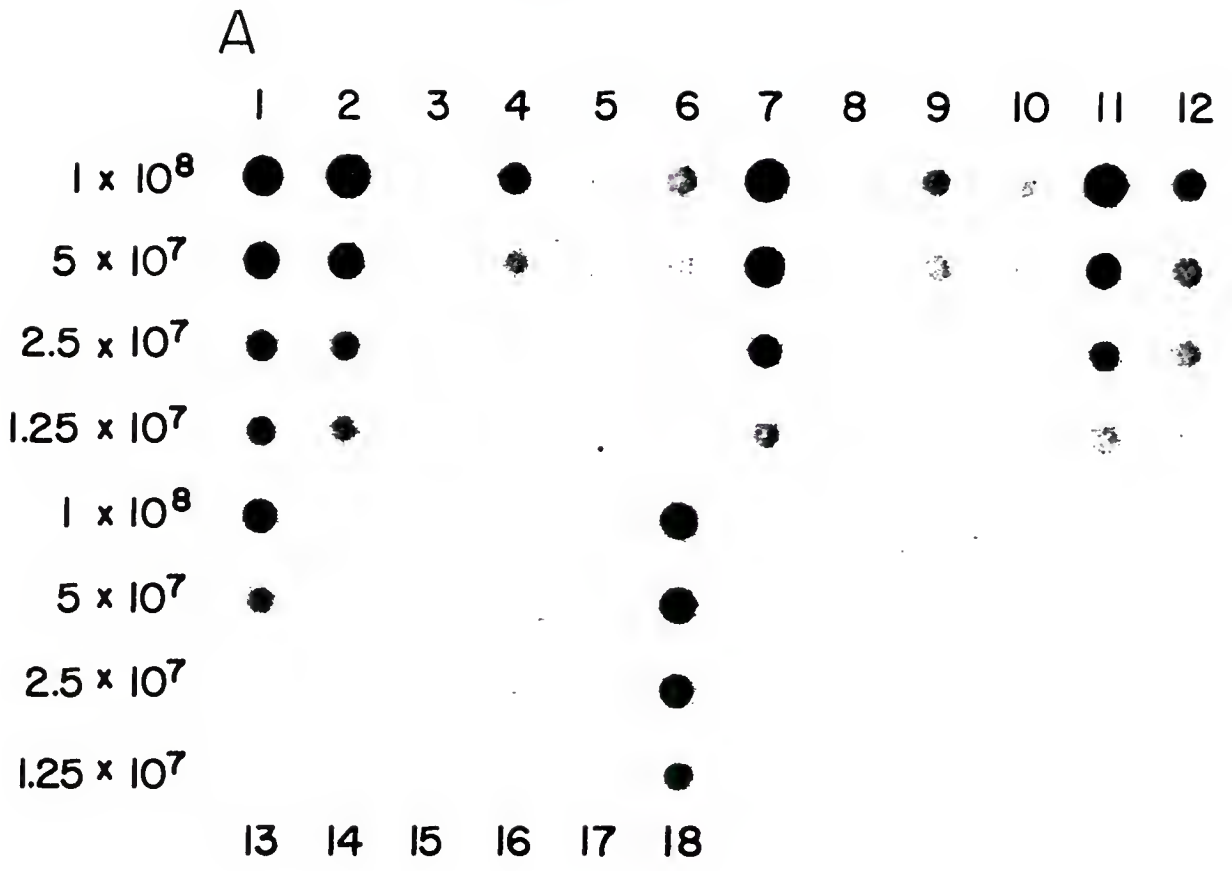
	Strain	M-type
1.	B920	49+
2.	B512	4
3.	11434	12+
4.	A992*	18
5.	A995	57+
6.	A207	2
7.	A928	55+
8.	B281	12+
9.	A547	NT
10.	B931*	2
11.	B905	2
12.	A374	12+
13.	B438	18
14.	D897*	12+
15.	B923	12+
16.	B915	49
17.	F2030	1
18.	B515	NT

Panel B

	Strain	M-type
1.	646	49+
2.	647	49+
3.	648	1
4.	650	22
5.	652	NT
6.	653	"787"
7.	654	11
8.	SHS1	NT
9.	SHS4	12+
10.	SHS7	NT
11.	SHS8	NT
12.	SHS9	NT
13.	SHS10	NT
14.	SHS14	NT
15.	SHS16	1
16.	SHS17	12+
17.	SHS18	12+

NT - Non-typable
\+ - Nephritogenic M serotypes (Stollerman, 1971)

Panel A contains fifteen nephritogenic strains and three non-nephritogenic strains (*). Autoradiography was at -70°C for 3 days with an intensifying screen.
Panel B contains seventeen non-nephritogenic strains. Autoradiography was for 1 day at -70°C with an intensifying screen.


A
1 2 3 4 5 6 7 8 9 10 11 12
1 x 10^8
5 x 10^7
2.5 x 10^7
1.25 x 10^7
1 x 10^8
5 x 10^7
2.5 x 10^7
1.25 x 10^7
13 14 15 16 17 18

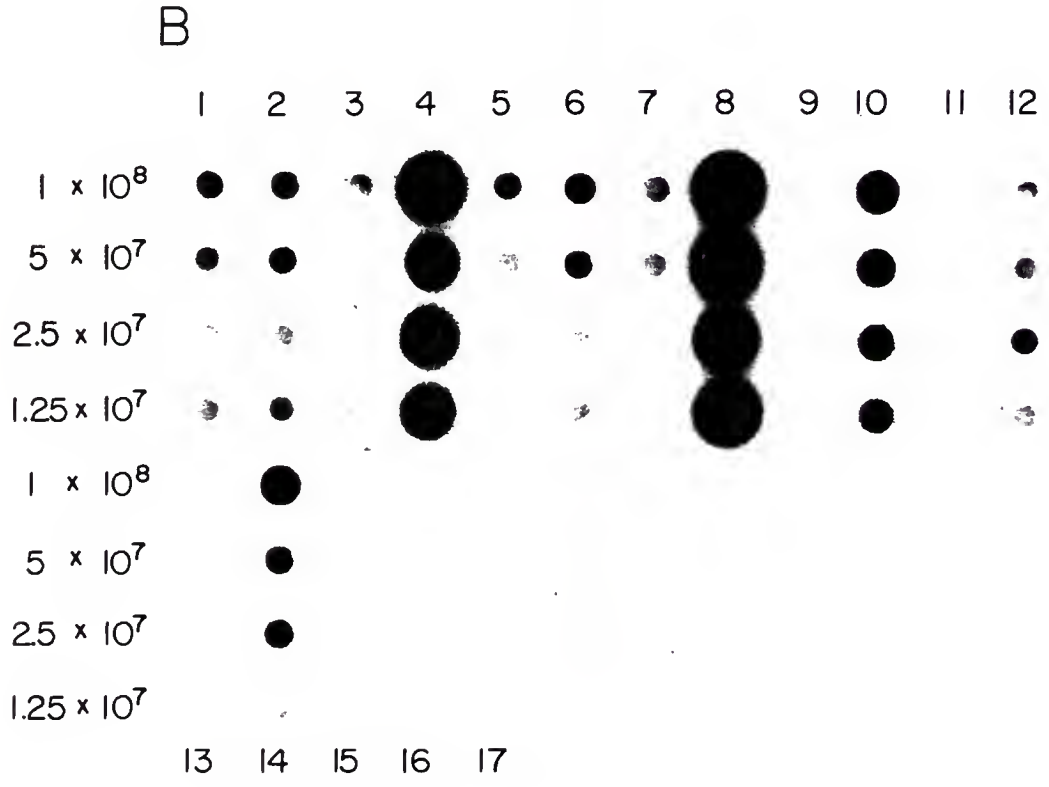

B
1 2 3 4 5 6 7 8 9 10 11 12
1 x 10^8
5 x 10^7
2.5 x 10^7
1.25 x 10^7
1 x 10^8
5 x 10^7
2.5 x 10^7
1.25 x 10^7
13 14 15 16 17

TABLE 4-1

Binding of ^{125}I Human IgG to Nephritogenic and Non-Nephritogenic Group A Streptococci

A

	1	2	3	4	5	6	7	8	9	10	11	12
1×10^8	883	1013	516	713	597	600	1083	597	618	612	901	689
5×10^7	736	799	562	614	549	565	858	546	609	578	689	630
2.5×10^7	672	660	516	524	577	537	723	488	542	550	624	624
1.25×10^7	651	608	531	495	517	523	585	500	529	520	557	566
1×10^8	658	568	558	554	550	836		512				
5×10^7	608	511	572	542	571	753		495				
2.5×10^7	549	503	531	536	535	709		512				
1.25×10^7	548	552	552	515	538	652		504				
	13	14	15	16	17	18		Con-trol				

TABLE 4-1 continued

B

	1	2	3	4	5	6	7	8	9	10	11	12
1×10^8	1299	1349	1002	14421	1103	1638	935	20763	588	3747	595	899
5×10^7	1208	1299	880	7013	916	1244	925	21088	632	2789	642	1002
2.5×10^7	1000	991	743	10830	734	885	725	14264	623	2322	641	1242
1.25×10^7	1088	1231	840	7044	762	1042	820	15782	599	2257	613	969
1×10^8	711	3332	759	677	778		585					
5×10^7	624	1474	766	680	603		602					
2.5×10^7	732	720	683	1679	618		604					
1.25×10^7	653	1092	676	652	610		547					
	13	14	15	16	17		Con-trol					

Each nitrocellulose blot was cut into sections that contained individual dots. Each section was counted using an LKB autogamma counter.
A - CPM from the blot in Fig. 4-1A
B - CPM from the blot in Fig. 4-1B

Since the results obtained in Chapter Three indicate the presence of at least two distinct Fc receptors on group A strains, the experiments described above using ^{125}I labeled human IgG as probe were repeated on the nephritogenic strains with each ^{125}I labeled human IgG subclass. The results are presented in Table 4-2. In general, the nephritogenic strains which were able to bind the ^{125}I labeled human IgG probe, showed no particular preference for any one subclass. One strain, A547, did bind IgG_3 to a greater extent than any other subclass, while another strain, A928, had opposite binding characteristics. These two strains might be useful in isolating the type IIb and type IIa Fc receptors, respectively (see Chapter Three).

Discussion

The role of streptococcal products in generating post-streptococcal glomerulonephritis is not well understood. In 1982, Boyle proposed that the Fc receptors and the M protein were critical components and proposed the following hypothesis which contains these elements:

1) Nephritogenic strains of streptococci produce an Fc-reactive protein.
2) This protein is released alone, or associated with other cell wall constituents and forms complexes with normal IgG which efficiently bind and activate complement.
3) These complexes of Fc-reactive protein and IgG are either not cleared or inefficiently cleared by the reticuloendothelial system and lodge in the kidney. The presence of the anti-phagocytic M protein within such a complex could inhibit its efficient clearance.

TABLE 4-2

Interaction of Nephritogenic and Non-nephritogenic Group A Streptococci with Human IgG Subclasses

	STRAIN	M-TYPE	TOTAL IgG	IgG_1	IgG_2	IgG_3	IgG_4
1.	B920	49	++++	+++	++	++++	++
2.	B512	4	++++	+++	+++	++	+++
3.	11434	12	+/-	-	+/-	-	+/-
4.	A992*	18	++	+	+/-	++	+
5.	A995	57	+	-	+	-	+
6.	A207	2	+	++	+	+/-	+
7.	A928	55	++++	+++	+++	+/-	+++
8.	B281	12	+/-	-	+/-	-	+/-
9.	A547	NT	++	-	-	++++	+/-
10.	B931*	2	+/-	-	-	-	-
11.	B905	2	++++	++	+	++++	+
12.	A374	12	++	+/-	-	+	-
13.	B438	18	+++	++	+/-	++	+
14.	D897*	12	+/-	-	-	-	-
15.	B923	12	+	-	+/-	-	-
16.	B915	49	+/-	-	-	+	-
17.	F2030	1	+/-	-	-	-	+/-
18.	B515	NT	++++	+++	+++	++	+++

* = non-nephritis causing strains
NT = nontypable strain
- = no detectable binding
+ = binding detectable with 1×10^8 bacteria
++ = binding detectable with 5×10^7 bacteria
+++ = binding detectable with 2.5×10^7 bacteria
++++ = binding detectable with 1.25×10^7 bacteria

4) Once in the kidney, these complexes are trapped in the vicinity of the basement membrane and tissue damage results both through the action of complement itself and by other components of the host's cellular immune system recruited by chemotactic split products of complement (e.g., C5a).

This hypothesis is summarized in Fig. 4-2.

To test the first element of this hypothesis, a rapid dot-blotting procedure was developed which enabled me to compare the Fc receptor expression on nephritogenic vs. non-nephritogenic group A streptococcal strains. Fifteen out of the eighteen nephritogenic strains tested (73%) were positive for Fc receptor expression (Fig. 4-1A), whereas 65% of the non-nephritogenic strains also had Fc receptors on their surface (Fig. 4-1B). Since the nephritogenic strains tested were library strains, the possibility exists that these strains have lost Fc receptor expression during subculture (Kronvall, 1973a; Christensen and Oxelius, 1974; Freimer et al., 1979). Although the majority of non-nephritogenic stains tested were fresh isolates, non-nephritogenic library strains were also tested and 71% of these library strains still maintained Fc receptor expression.

In Chapter Three, two distinct Fc receptors were isolated from a group A streptococcal strain. One Fc receptor was capable of binding human IgG subclass 1, 2, and 4, while the other Fc receptor was specific for human IgG subclass 3. Lewis et al. (1970) have reported that the immunoglobulin subclass composition of the glomerular deposits in human renal diseases was selective and did not reflect the normal serum concentration of these proteins. Patients who had granular deposits of immunoglobulin, which suggest an immune complex

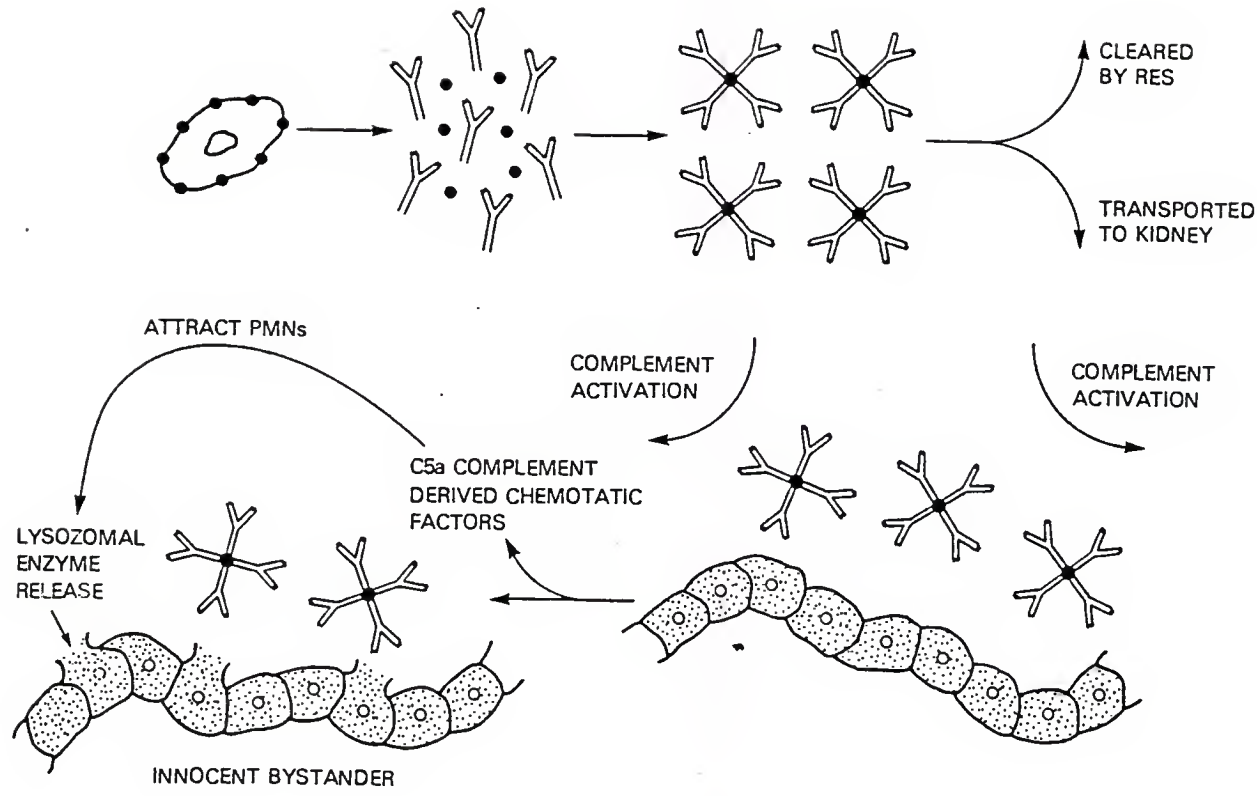


Fig. 4-2. Proposed mechanism of the pathogenesis of post-streptococcal glomerulonephritis.

pathogenesis, tended to have selective deposits of immunoglobulin composed of a single or dominant subclass, usually IgG_2 (Lewis et al., 1970). In light of these studies, the nephritogenic group A strains were tested for reactivity with each human IgG subclass (Table 4-2), however, none of the Fc receptor-positive nephritogenic strains showed selective binding to any particular human IgG subclass.

Since Fc-reactive proteins were found on the majority of both nephritogenic strains and non-nephritogenic strains, these results indicate that nephritogenicity probably requires other factors in addition to the ability to produce Fc-reactive proteins. One factor which has been shown to be important to this pathogenic process is the anti-phagocytic M protein (Jacks-Weis et al., 1982). The presence of the M protein in these complexes might prevent their clearance from the circulation. Such uncleared complexes could lodge in the kidney, activate complement, and initiate the pathogenic process that leads to kidney destruction. By contrast, complexes lacking this protein, e.g., staphylococcal protein A - IgG complexes, would be effectively cleared from the circulation and consequently an absolute correlation between Staphylococcus aureus infections and nephritis would not be expected.

Certain M types of group A streptococci are correlated with nephritogenic potential (Stollerman, 1971), however, not all group A streptococci with those nephritogenic serotypes cause glomerulonephritis. Also, post-streptococcal glomerulonephritis is not always caused by a strain with a nephritogenic M serotype. In these studies, only 53% of the strains isolated from patients with glomerulonephritis had a nephritogenic M serotype. Of these strains, 63% had Fc receptors on their surface. These results are similar to those obtained from the

strains isolated from patients without glomerulonephritis, in which 65% of the strains have Fc receptors on their surface. Consequently, the results of this study suggest that no absolute correlation exists between Fc receptor expression and nephritogenicity. Although both the M protein and Fc receptors have been proposed as virulence factors (Ginsberg, 1972; Schalen, 1982; Christensen et al., 1977,1978,1981), more studies need to be done in order to determine all the factors required for post-streptococcal glomerulonephritis. Villarreal et al. (1979) has reported the occurrence of an extracellular protein isolated from patients with post-streptococcal glomerulonephritis, however, this protein, like the type II Fc receptor and the M protein, was sometimes produced by streptococci obtained from patients without the disease. No biological properties of this extracellular protein could be determined, but based on its reactivity with normal and specific rabbit antiserum, its properties were distinct from those of the type II Fc receptor (Ohkuni et al., 1983).

The dot-blotting procedure described in this chapter can be applied to the study of other bacterial diseases. In a recent study, I have applied this technique to study the expression of a variety of different receptors on clinical bacterial isolates recovered from patients suffering from endocarditis (Yarnall et al., 1985). Receptors for collagen type I and type III, Clq, fibrinogen, fibronectin, and human IgG were studied. The results of this study failed to identify a common surface receptor that could account for the ability of these bacteria to colonize damaged heart tissue.

Since the early studies of Koch (1903), it has been necessary to fulfill a certain number of criteria before it is possible to establish

a cause-effect relationship between any infectious agent and a disease process. In carrying out the studies reported in this chapter, I was aware that it would not be possible to evaluate critically the hypothesis summarized in Figure 4-2. The results I have obtained would indicate that the immunoblotting technique I have developed can be used as an efficient approach for more fully characterizing individual strains of bacteria isolated from patients with a specific disease. While this approach will never lead to elucidating a definitive pathogenic mechanism, the information obtained from these studies is helpful in determining if any correlation exists between a particular receptor or surface protein and an infectious agent isolated from individuals with a common disease state.

CHAPTER FIVE
CONCLUSION

Bacterial Fc receptors have been known for several years. Myhre and Kronvall (1981) have characterized the functional activity of these receptors on the bacterial surface, identifying five basic types. The type I staphylococcal Fc receptor (Langone, 1982a) and the type III group C streptococcal Fc receptor (Reis et al., 1984c,1984d,1985; Bjorck and Kronvall, 1984) have been purified and extensively characterized. Little information is available on the other three types of Fc receptors. A type II Fc receptor which is found on certain strains of group A streptococci has been isolated (Grubb et al., 1982), but with low yield, and the functional activities of the isolated type II Fc receptor were not characterized.

In this study, I have described the isolation and characterization of the type II Fc receptors from a mouse-passaged group A streptococcus. A method was developed which enabled me to select an individual Fc receptor-rich substrain from which to isolate the type II Fc receptors. The type II Fc receptors were recovered in high yield and were composed of two molecular weight forms that were antigenically related, but functionally distinct. The 56,000 dalton receptor was capable of binding human IgG subclasses 1,2 and 4, pig, and rabbit IgG. The 38,000 dalton receptor could only bind the Fc region of human IgG subclass 3. This is the first report of a unique receptor for a particular subclass of human IgG.

The isolation of an Fc receptor which binds the Fc region of human IgG_3 has several practical applications. It can be used for separating or depleting IgG_3 from serum or secretions by immobilizing the Fc receptor on sepharose. The IgG_3-specific Fc receptor can be radiolabeled or enzyme-linked for use in assays to detect and quantify IgG_3. This would be beneficial in diagnosing diseased states in which the production of an IgG subclass is restricted to IgG_3. For example, Beck (1981) observed that antibodies directed against the rubella virus were primarily of the IgG_3 subclass. Antibodies against thrombocytes in the serum of patients with idiopathic thrombocytopenic purpura were demonstrated by Karpatkin et al. (1973) to be limited to IgG_3. In addition, an extensive study by Natvig et al. (1967) using Gm-specific antisera showed that the Rh antibodies in the sera of mothers after an incompatible pregnancy belong to the IgG_3 subclass. The IgG_3 Fc receptor might be useful in exploring the mechanism of the IgG_3 restriction in these diseases.

The role of group A streptococcal Fc receptors in the pathogenesis of infection or post-infection sequelae is not clear. The distribution of the Fc receptors on nephritogenic and non-nephritogenic group A streptococci was studied, but no absolute correlation could be established. The biological activities of the type II Fc receptors can now be explored using the purified Fc receptors in both in vivo and in vitro systems. Complement activation, mitogenesis, and the nature of Fc receptor-IgG complexes can be examined to clarify the role of Fc receptors as virulence factors.

The methods described in this study to detect secreted and cell associated Fc receptors, and to solubilize Fc receptors can be used in

future studies to explore new directions. First, streptococcal strains can be treated with various mutagens or antibiotics to create a strain which can secrete Fc receptors. Methicillin has been used to isolate strains of Staphylococcus aureus that produce only extracellular protein A (Winblad and Ericson, 1973). An enhancement of the formation of extracellular protein A was also achieved by growing Staphylococcus aureus in the presence of puromycin (Movitz, 1976). These approaches can be applied to streptococci to produce a mutant strain which secretes Fc receptors.

With the discovery of a unique receptor for the Fc region of IgG_3, it is possible that different strains of streptococci have Fc receptors that are specific for other human IgG subclasses. Using the techniques described in Chapter Three, one can search for new unique Fc receptors. Identification of Fc receptors for each human subclass would be of great value in identifying and isolating IgG subclasses for use in immunoanalytical and immunodiagnostic assays and for studying the fine structure of IgG constant domains.

Finally, the ability to screen large numbers of individual bacterial colonies would be useful in identifying clones carrying a specific gene that codes for surface receptors or proteins. Isolation of a bacterial vector with the gene for a group A streptococcal Fc receptor could be accomplished using this technique. Once the gene is identified and cloned, several studies could be done to clarify the loss of Fc expression observed on subculture. Also, specific mutations of the cloned gene could determine if group A streptococcal Fc receptors are virulence factors and whether or not they are involved in the pathogenesis of post-infection sequelae.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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